TITLE OF THESIS

Measurement of mechanical properties of the skin in lower limb chronic venous disease compared to established non-invasive methods of assessment.

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ABSTRACT

Chronic venous disease (CVD) of the lower limbs is a major problem in the western world with 1% of the adult population estimated to be affected at any one time. The clinical sequelae of CVD of the lower limbs range from oedema, haemosiderosis and pigmentation, to gross lipodermatosclerosis (LDS) and venous ulceration. The site most commonly affected is the gaiter area of the lower limb. The extent and severity of venous disease can be assessed by clinical and physiological methods which include duplex ultrasonography and plethysmography. Tissue oedema can be assessed by volumetric or circumferential measurements and venous ulcers may be quantified by area measurements and response to treatment in ulcer healing studies. In the vast majority of patients a spectrum of skin changes precedes venous ulceration. At present, there is no standardised objective method of assessing the degree of skin change in these patients, so that the response to treatment can be objectively monitored .

I have developed a tissue tonometer and standardised the methodology for the objective assessment and quantification of the skin changes seen in patients. The tissue tonometer is a simple non-invasive instrument which uses a sensing device that detects the movements of a loaded plunger placed on the skin. The movement of the plunger is dependent on the mechanical properties of the skin and subcutaneous tissue. The instrument is positioned on the gaiter region of the leg with the subject in the supine position. The movement of the plunger into the tissues is recorded and analysed by a computer. The data obtained from the tonometer were analysed as distance and rate constant parameters. A simple mathematical model using spring and dashpot constants was also applied to see if it fitted the data.

Skin compliance was investigated in normal control subjects and patients with varying severity of skin changes due to CVD, clinically classified according to the CEAP (**C**linical, (A)**E**tiological, **A**natomical and **P**athophysiological) method. There was a significant reduction in skin compliance in patients with clinically severe LDS as compared to normal controls and patients with pigmentation alone or oedema without any clinical evidence of skin change.

I further investigated the correlation between the recently introduced CEAP method of classification and scoring of chronic venous disease of the lower limbs with the tissue tonometry findings and parameters obtained with duplex ultrasonography, air plethysmography and photoplethysmography.

Tissue tonometry provides a standardised objective means of assessing the severity of skin change in CVD which may prove to be useful in evaluating response to a particular treatment and comparing data from different centres. The deterioration of the venous physiology shown by blood flow measuring techniques correlates poorly with the clinical sequelae of venous disease, whether assessed by a trained observer or measured by the tonometer. Patients show a wide range of sensitivity to venous valvular incompetence, suggesting that factors related to the tissue response to venous hypertension are crucial in determining which patients develop venous ulceration.

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DECLARATION

This thesis is entirely my own work carried out with the assistance of my supervisor, Mr PD Coleridge Smith.

Neither this thesis, nor any part of it, has been submitted before to this or any other university in consideration for a degree.

Some of the work described in this thesis has been presented before learned societies (see appendix I).

I agree that, the libraries of London University and University College London Medical School may make this thesis available for public reference, inter-library loan and copying.

ACKNOWLEDGEMENTS

First of all, I would like to thank Mr PD Coleridge Smith, my supervisor, for all his patience and assistance in guiding me through this thesis especially in the development of the tissue tonometer and its software.

I would also like to thank Professor M Hobsley (previous head of Department of Surgery, UCL Hospitals) and Professor I Taylor (current head of Department of Surgery, UCL Hospitals) for allowing me to carry out this work in the department of Surgery.

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I wish to thank all my colleagues and friends for their genuine support and assistance throughout, especially Mr S Aly, Mr S Shoab, Mr S Sarin, Mr A Abu-Own, Mr D Shields, Mr M Saharay, Miss L Boden, Mrs B Lambourne, Miss K Sommerville, Miss C Butler and Dr N Riazuddin.

Most of all, I would like to thank Caroline Munklinde for helping me with the illustrations and for her continuous support.

And finally, I would like to thank my parents and family, for without them, this would not have been possible.

FOREWORD

'When you can measure what you are speaking about and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the edge of science, whatever the matter may be'.

Lord Kelvin, 1891

(From: Thomson W: Popular lectures and addresses, ed 3. Vol. 1. Constitution of matter. London, 1891, Macmillan and Co.).

Dedicated to the memories of auntie Farida and auntie Layah.

SECTION I

Introduction

1.1 Epidemiology of lower limb chronic venous disease.

Lower limb chronic venous disease (CVD) has been recognised since ancient times. The clinical sequelae of lower limb CVD, which include oedema, liposclerotic skin changes and ulceration, occur in between 3% (Coon et al, 1973) and 11% of the population (Da Silva et al, 1974). It has also been estimated that the annual development of new symptoms is approximately 1% for oedema and 0.8% for skin changes (Widmer et al, 1992). The annual incidence of varicose veins in the female population averaged at 2.6% and 1.9% in males (Brand et al, 1988). In the western world about 0.3% of the adult population have active venous ulcers (Callam et al, 1985; Cornwall et al, 1986; Nelzen et al, 1991; Coon et al, 1973) and the prevalence of active and healed ulcers combined is about 1% (Nelzen et al, 1991). The point prevalence estimates of ulcers present in the community varies from 0.11% in Australia (Baker et al, 1992), to 0.15% in Scotland (Callam et al, 1985), 0.18% in England (Cornwall et al, 1986) and 0.30% in Sweden (Nelzen et al, 1991). From a population questionnaire sent to 549 elderly people in Scotland it was estimated that the overall prevalence of lower limb ulceration was 3.6% among people older than 65 years. This corresponds to 1% of the adult population in Scotland (Dale et al, 1983). In the United States of America, the Tecumseh community health study, a longitudinal study of a population in Michigan, extrapolated their data to the United States 1970 consensus figures and estimated that in the USA, 24 million people have significant varicose veins (prevalence 12.9%), while 6 to 7 million people have stasis changes in the skin of the legs (prevalence 3%) and that nearly half a million people have or have had a varicose ulcer (prevalence 0.1%) (Coon et al, 1973).

Leg ulcers can have a variety of causes with the majority being as a result of chronic venous disease. In a cross-sectional population study in a health district in England, a random sample of 100 patients (193 limbs) with leg ulcers were examined with ultrasound and photoplethysmography to assess the venous and arterial circulation. It was found that 38% had deep venous incompetence, 43% had superficial venous incompetence, 22% was of mixed venous and arterial aetiology and 9% was of arterial aetiology alone. (Cornwall et al, 1986). Leg ulcers with liposclerotic skin changes were most commonly found in the gaiter area of the limb. One study showed that 88% of ulcers were located in the gaiter area of which 76% were on the medial side (Callam et al, 1992).

It has been reported that patients of low social class have delayed ulcer healing (Franks et al, 1995). In general, the prognosis of leg ulcers is poor with only 50% healing at 4

months (Skene et al, 1992), 20% remain open at 2 years and 8% at five years (Callam et al, 1987).

The effects of lower limb chronic venous disease can also have a huge impact on the national economy of the country. In one study, 12.5% of patients with ulcers took early retirement (DaSilva 1992) and it was estimated that in England and Wales, about 500,000 working days are lost per year. In the USA, the figure is 2 million working days (Browse et al, 1978). In Brazil, the fourteenth most frequently quoted disease for absenteeism from work and the thirty second most frequent cause of permanent disability and financial aid is lower limb chronic venous disease (De Castro-Silva, 1997).

In France, the total annual cost of venous insufficiency to the health service in 1991 was estimated to be 14.7 billion French francs (Lafuma et al, 1994), representing 2.6% of the whole health expenditure. In the United Kingdom, it is estimated that the annual cost of venous ulcers is between 400-600 million pounds sterling (Callam et al, 1992; Jantet et al, 1992) and in the United States of America, the figure is over 1 billion dollars (Hume et al, 1992).

1.2 A brief historical review of lower limb venous disease.

The problems of the treatment of lower limb venous disease is not a new one and has been recognised since ancient times. The *Ebers Papyrus*, named after the German professor Georg Ebers (1837-1898), described possibly the first 'venous' publication from around 1550BC in which varicose veins were described as *'serpentine windings'* in ancient Egypt. In the 4th Century BC in Athens, the oldest known illustration of a varicose vein, a stone plaque showing a man holding a leg with a varicosity of the long saphenous vein, was found at the foot of the acropolis in Athens in the shrine of a physician named Amynos. Between 460-377BC, Hippocrates in his work, *De ulceribus*, documented a connection between leg ulcers and varicose veins and that the leg ulcers contained blood and *'evil humours'*. Hippocrates is also said to be the first to note an association between the two and recommended incision of the varices and compression, to squeeze out the 'humours'.

During the middle ages, there was still a widespread belief that as a result of standing, 'evil humours' would collect in the legs and cause ulcers. Avicenna in AD900 still supported the Hippocratic view that ulcers should not be allowed to heal because they were a site from which 'evil humours' could escape. If an ulcer did heal, he advised that

it should be deliberately broken down again. Some thought that it was dangerous to apply compression bandages which would prevent the 'humours' from escaping thus causing melancholy and madness.

Throughout the Middle Ages, the dogmatic views of the Church with regards to access to post mortem human specimens, marked an enduring stagnation in the development of surgery in Europe. The relaxation of the Church's ban on human dissection paved the way for the anatomists with Leonardo da Vinci (AD1452-1519) producing over 779 anatomical drawings many of which illustrated the venous system. The famous anatomist Vesalius (AD1514-1564) described the venous system in great detail but could not find the valves alluded to by Canano in AD1546. However, in 1551, Amatus stated that veins contained valves possibly the first description of a venous valve. Hieronymus Fabricius of Aquapendente re-described the valves in his work entitled *De venarum ostiolis* after demonstrating them at public dissections in 1579. In 1585, Salmon Alberti published the first recorded drawing of a valve in a vein. He noticed that they stopped retrograde flow but thought that they participated in the control of the ebb and flow of blood described earlier by Galen (AD130-200).

The belief that 'humours' were responsible for causing ulcers was still around in 1620, when Fallopio stated that varicose veins caused ulcers because the *'varices carry faeculent humours which cause ulceration'.*

In 1628, the greatest physiological revolution in the history of medicine which provided the foundation of our present understanding of the circulation, was published by William Harvey in the book *Exercitatio anatomica de motu cordis et sanguini's in animalibus.* Biohistorians agree that the landmark work done previously by Fabricius on venous valves led directly to William Harvey's discovery of the circulation. It can also be said that the background for Harvey's discoveries may have been laid as early as 1550BC in the *Ebers papyrus*. Harvey demonstrated that the blood circulated and made the fundamental observation that the valves in the veins were there to ensure unidirectional flow of blood. After William Harvey's description of the physiology of the venous circulation and the function of the valves in the veins, the belief that 'evil humours' were responsible for ulcers were finally cast aside.

The principles of modern day treatment of varicose veins and venous ulcers have not changed much since the time of Hippocrates with the use of compression treatment and/or varicose vein surgery being the recommended methods of healing ulcers, but the technology has improved. In the last three decades, there has been a large number of

publications on the treatment of venous disease and the improving of surgical techniques, surgical instruments, compression treatment techniques and materials. However, with better understanding of the microcirculation several authors have suggested new theories of the causes of venous ulcers. Amongst them are the fibrin cuff theory (Browse and Burnand, 1982) and more recently, the white cell trapping theory (Coleridge Smith et al, 1988). It is hoped that the latest investigations of the microcirculation will provide a better understanding of causes of venous ulcers and may one day provide a cure by pharmacological means.

1.3 A brief historical review of the development of venous testing.

There has been a gradual development of clinical tests of venous physiology since the latter part of the 17th century after the William Harvey publications which shed a new light on the venous circulation and the function of the venous valve. All of these were dependent upon careful clinical observation by an experienced physician interested in the physiological function of the superficial and communicating venous valves and the interplay of the muscle pump. Fabry in AD1589 while examining a leg ulcer, described blood in the veins receding immediately towards the head when the leg was elevated and descending instantly when the leg was placed in a dependent position, thus describing what we now know as venous reflux or retrograde flow in the veins. Sir Benjamin Brodie, in 1846, formalised that observation by describing his test of venous valvular function where, after having emptied the veins of the leg, he demonstrated that reflux could be controlled by pressure on the vein above. Trendelenberg in the 1890s popularised this test and is still a standard clinical test. Perthes in 1895 was the first to describe a test to assess muscle pump function by asking the patient to walk after proximally constricting the superficial veins and noting that the superficial varicosities would disappear only if the valves of the deep venous system were competent.

The tests mentioned above have been very important in the application of modern electronic equipment to assess venous pathology. Today, we use the powers of continuous-wave, pulse-wave and colour Doppler instruments to assess blood flow based on the powers of observations that had been made previously in the tests described above.

1.4 Recent advances in the non-invasive diagnostic techniques of venous testing.

In the 1970s, several non-invasive methods for the study of peripheral vascular and cerebrovascular problems were developed. These include Doppler ultrasound, lower extremity pressures, pulse volumes, oculoplethysmography and carotid phonoangiography for carotid bifurcation studies and impedance mercury strain-gauge and air plethysmography for study of venous physiology. These measurements of velocity, pressure and volume, represented the foundation of methodology in the vascular laboratory.

In the 1980s, the most important technical advances in instrumentation were the development of pulsed Doppler systems and their use in conjunction with B-mode imaging in the duplex scanner, which will be described in more details later in the chapter.

By 1985, duplex scanning was the main direct test for disease of the carotid bifurcation and had replaced most of the indirect tests including bruit analysis, oculoplethysmography, and periorbital Doppler velocity studies, because duplex scanners provide anatomical and functional information about the critical carotid lesions.

Since 1985, several new instruments have been developed. The most significant is real-time colour Doppler imaging (see section 1.4.3). Venous applications of the colour duplex ultrasound scanner, both for acute peripheral venous thrombosis, pre-operative mapping of veins and for identifying and quantifying chronic venous reflux, have defined a new era of non-invasive studies.

It is now possible to derive precise functional information regarding the venous system from non-invasive tests using Doppler and plethysmographic techniques without the need of exposing the patients to ionising radiation, blood products or even skin puncture, all of which may subject the patient to risk. These non-invasive investigations are very well tolerated by patients and can be used in sequential studies for repeat measurements in clinical studies.

1.4.1 Air plethysmography

This is a pneumatic plethysmograph designed for measuring absolute volume changes in millilitres or millilitres per second in the lower and upper extremities by detecting volume changes which occur in response to postural changes and exercise. These detected volume changes are analysed to obtain parameters of venous function and

arterial perfusion. In the modern version a plastic sleeve is zipped around the patient's leg and filled with air to a pressure of 6-8mmHg known as the 'bias' pressure (Christopoulos et al, 1988). This is sufficient to ensure close conformity with the leg, but does not interfere with the venous physiology under study. It is now possible for the device to be calibrated so that absolute volume changes in the limb can be deduced (see section 2.2 for details).

This technique has many advantages in that it is simple, non-invasive, quantitative, has multiple applications and can be calibrated during each examination while connected to the subject. This technique has been validated and shown to measure global reflux in ml/second (Christopoulos et al, 1987; Katz et al, 1991).

1.4.2 Photoplethysmography

The photoelectric plethysmograph was first described in the 1930s and was intended for use in the assessment of the arterial system rather than the venous system where it was first applied in 1978 (Barnes et al, 1978). It consists of a sensor combining a light emitting diode (LED) operating in the near infrared and a very sensitive phototransistor detector. Light emitted by the LED is reflected from blood moving in the area being investigated. Changes in the reflected signal are detected by the phototransistor and processed to display the waveform. Photoplethysmography provides a sensitive means of detecting blood flow in the microcirculation near the skin's surface by assessing the variation in the light absorption of the skin by haemoglobin in the dermal venous plexuses. When these are full during high venous pressure the haemoglobin in the red blood cells absorbs light. As venous pressure falls the venous plexuses become less full and light transmission increases. The light is derived from a light emitting diode which emits non-coherent light with an emission peak at about 805 nanometres. The transducer is designed so that when applied to the skin with double-sided adhesive tape, light must traverse the skin to reach the detector. A phototransistor is used which also amplifies current produced by light falling on the sensitive region. The transducer is connected to an amplifier which permits adjustment of the output voltage. The resulting trace may be recorded by a chart recorder or displayed on a computer. The methodology is described in details in section 2.3.

1.4.3 Measurement of blood flow using the Doppler principle

In a Doppler ultrasound system, the difference in frequency between transmitted and received signals contains the blood flow information. The Doppler principle, the Doppler equation and the instrumentation of spectral analysis and its basic principles are described below.

Doppler principle and the Doppler equation

The Austrian physicist Christian Johann Doppler (1803-1853) demonstrated mathematically that the frequency of sound emitted or reflected from a moving object varies with the velocity of the object (Nayman et al, 1974) and is shown in the Doppler equation below. The frequency appears to increase to an observer who is moving towards the source of the sound and appears to decrease to an observer moving away from the source.

$$f_D = \frac{2 f v}{c} \cos \theta$$

where f_D is the change of the ultrasound frequency (the Doppler shift frequency),

f is the frequency of the incident sound wave,

v is the relative velocity between the transducer and the target,

c is the speed of sound in tissues (1.54 mm / μsec),

and θ is the angle of insonation between the direction of flow and ultrasound beam.

In 1957, Satomura first described a method of utilising this effect for detection of moving structures within the body (Satomura et al, 1957). The use of Doppler ultrasound as an indicator of blood flow was first suggested in the early sixties (Franklin et al, 1961).

Spectral Analysis Instrumentation

Sounds are created by the vibrations from objects, which travel through the air as sinusoidal waves with different frequencies. Sound waves are categorised as, infrasonic (less than 20Hz), audible (20Hz to 20kHz) and ultrasonic (greater than 20KHz).

In blood flow measurements using ultrasound, reflections from red blood cells rely on Raleigh-Tyndall scattering and it is rare to have a sample volume containing red blood cells travelling at a single velocity. Usually the signal received by the transducer is a

combination of many varied Doppler shifted frequencies. The Doppler signal is summation of information from a large number of reflectors or red blood cells with different velocities within the sample volume as selected with the pulsed Doppler gate (figure 1.4.3.1).

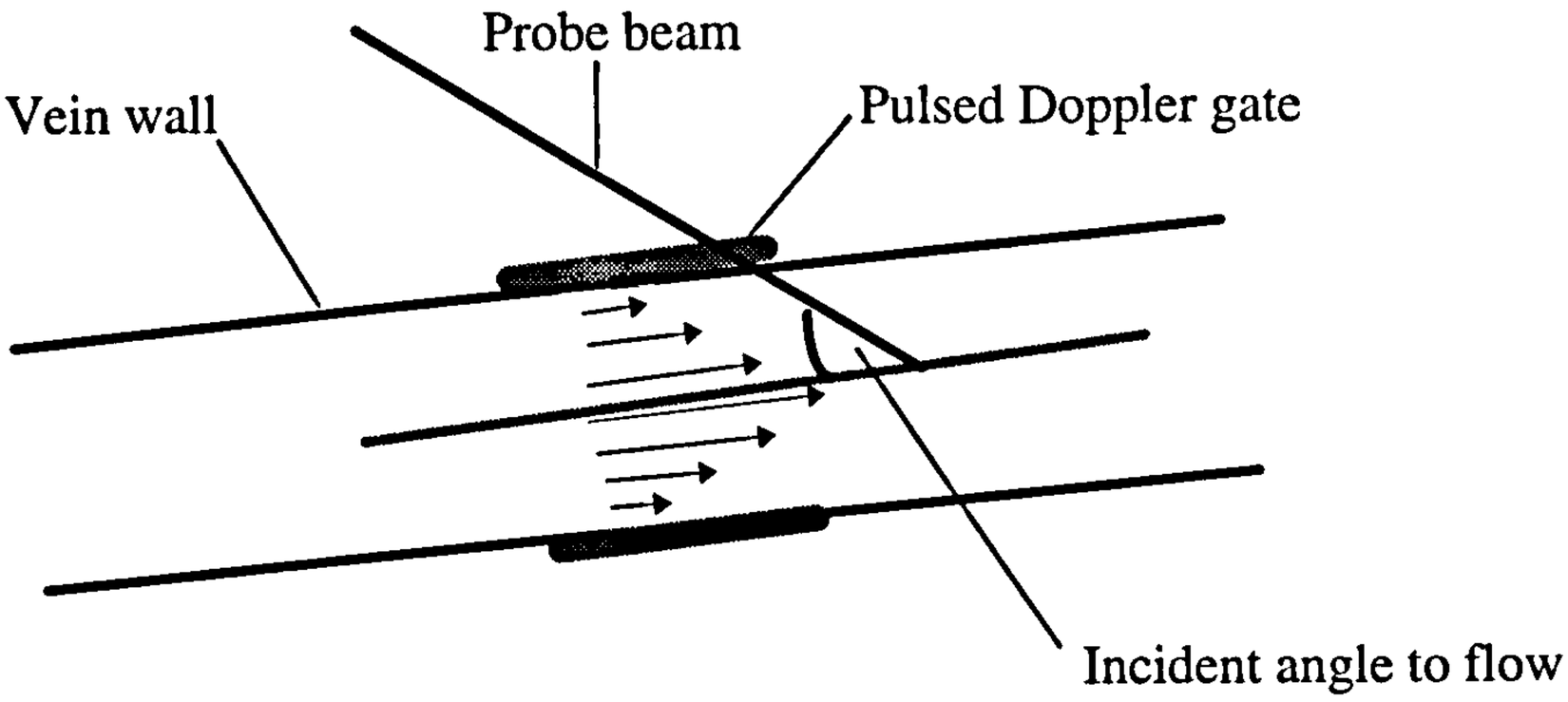

Figure 1.4.3.1 The pulsed Doppler gate should insonate the entire vessel wall with the incident angle to flow cursor parallel to the vessel walls and not exceeding 60 degrees. The slower velocity red blood cells are indicated by the smaller arrows with the fastest red cells indicated by a longer arrow in the middle of the vessel.

Spectral analysis is the breaking up of the components of a complex wave or signal and spreading them out in frequency order. The human auditory system accomplishes this for sound. The ear and brain break down the complex sounds we receive into the component frequencies contained in the sounds. Thus, we can listen to a Doppler signal and recognise normal and abnormal flow sounds. If all the blood cells were moving at the same speed and in the same direction, then a single Doppler shift will result. However, this is not the case and the extent of the range of generated Doppler-shift frequencies is dependent on the character of the flow. The fast Fourier transform is the mathematical technique that the instrument uses to derive the Doppler spectrum from the returning echoes of various frequencies. It is an algorithm which in real time analyses short periods of the Doppler signal into a series of spectra. A spectrum is a graph showing the relative power or amplitude of each of the velocity or frequency components in the Doppler signal. It is an amplitude versus frequency plot and is in effect a 'snapshot' of all the velocities in the sample volume at any instant in time.

Ultrasound imaging

Images are produced by examining the tissues with a scanning beam of ultrasonic sound which may be produced from a rotating transducer or a multielement phased array of transducers with no moving parts. The returning echoes carry information about the structure of the tissues and permit an ultrasound image to be reconstructed. Modern equipment allows this to be accomplished in real time, so that moving images of the structures beneath the transducer can be obtained as the examiner moves the transducer to examine different aspects of the tissues. Ultrasound is subject to Raleigh-Tyndall scattering which results in the absorption of energy by the tissues, as well as return of some of the transmitted sound. The scattering is proportional to the fourth power of the ultrasound frequency so that, as higher frequencies are used to improve resolution of structures within the tissues, greater difficulty is experienced in examining greater depths. Frequencies used to examine peripheral vessels are in the range 5-l0MHz. The structures can be identified and precise anatomy defined as demonstrated in the B-mode (brightness mode) image of a popliteal vein with the valve sinus and valve cusps clearly visible (Figure 1.4.3.2). Blood within veins often has a hyperechoic pattern, after a patient has been lying on the examination bed for a few minutes, which can be used to determine the direction of flow (Sigel et al, 1983; Machi et al, 1983). However, an imaging system alone cannot provide precise information regarding flow.

Duplex ultrasound imaging system

The limitation of having no flow data available has been overcome in duplex ultrasound systems by the inclusion of a pulsed Doppler facility (Taylor et al, 1987). In some systems this is provided by the addition of an extra transducer in the scanning head which provides Doppler information. In other systems phased array transducers are used to provide both image and Doppler information. The pulsed Doppler mode means that precisely defined areas can be studied to determine the velocity and direction of flow. This is invaluable in examination of the venous system. The presence of flow in major vessels can be established, and the location of venous reflux, for example, can be determined beyond doubt (Szendro et al, 1986). The lower limb vessels may be identified in turn, with the patient standing or lying on a tilted examination couch with the feet dependent. Forward flow can be produced in the calf by manual compression and the presence of forward flow and reflux assessed in each vein of the lower limb in turn.

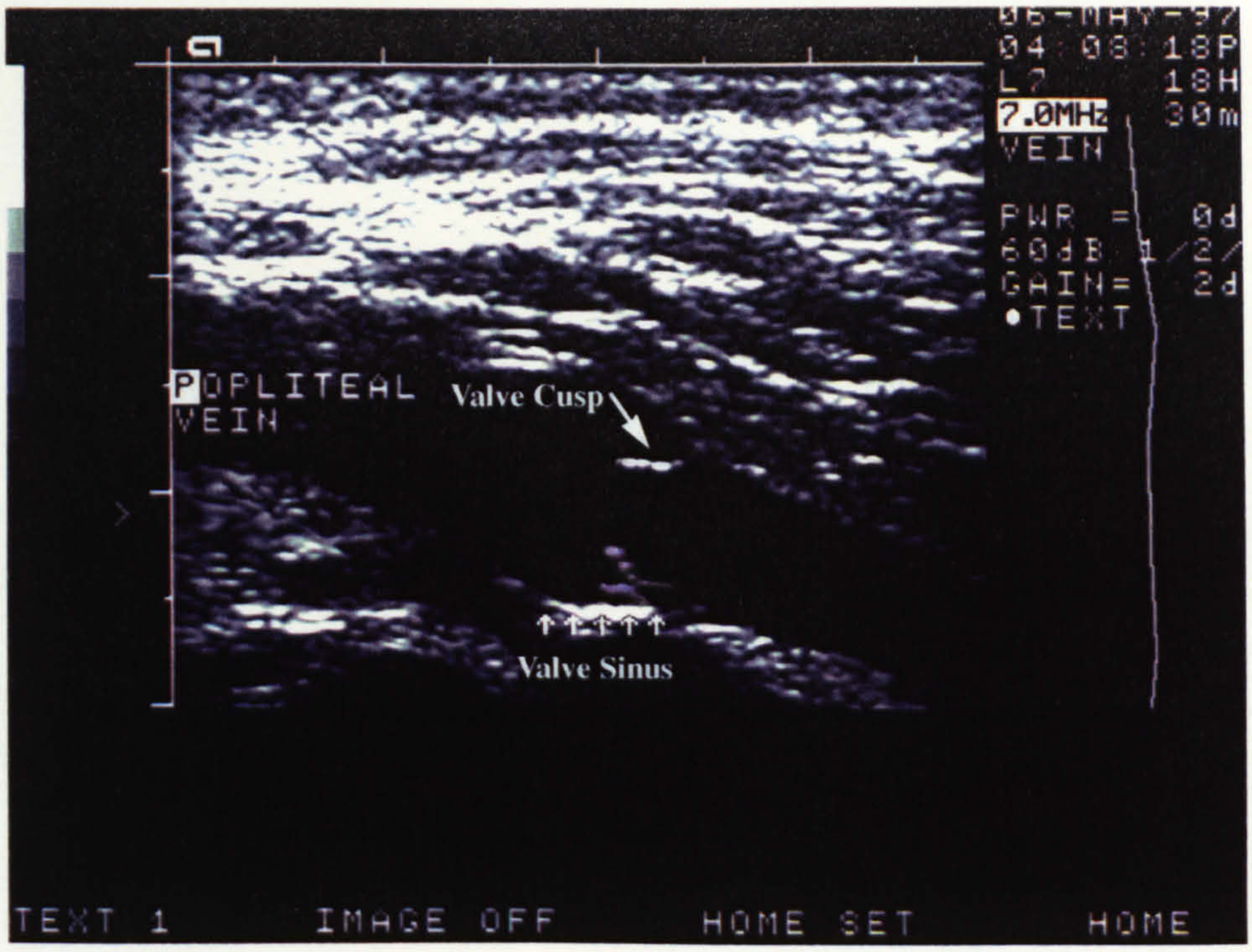

Figure 1.4.3.2 Brightness mode (B-mode) image obtained from a duplex scanner showing a popliteal vein with the dilated valve sinus and valve cusp easily identified.

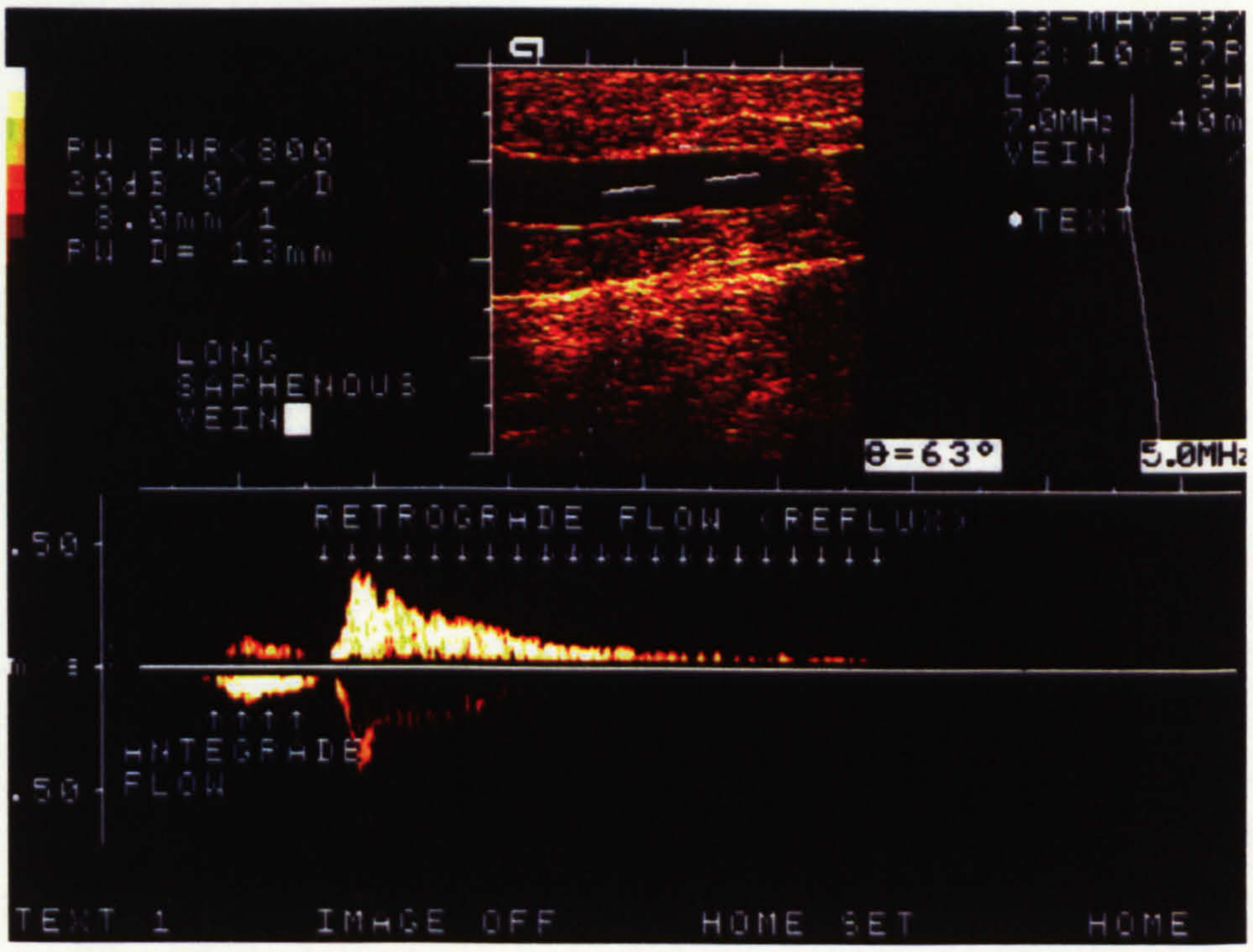

Figure 1.4.3.3 Brightness mode (B-mode) imaging combined with spectral Doppler analysis provides information of flow in the veins indicating antegrade flow (area below the baseline) and retrograde flow (reflux) (area above the baseline).

Virtually all veins below the inguinal ligament can be imaged completely by this technique and the competence of individual valves may be assessed (Rollins et al, 1987).

This technique allows the assessment of anatomical structures as well as defining function. Figure 1.4.3.3 shows a B-mode image of the long saphenous vein combined with spectral Doppler analysis to demonstrate antegrade flow and reflux in the vein after calf compression with the patient in the standing position.

Colour flow mapping

Duplex ultrasound imaging permits the assessment of each vein in turn, and localised flow anomalies may be overlooked. Colour flow ultrasound machines (triplex scanning, angiodynography) assess the Doppler shift of each pixel of the ultrasound image in a (user-defined) area and convert the usual grey scale image to a colour of a different saturation depending on the velocity of flow (Merritt et al, 1987). Distinct colours are used for forward and reverse flow so that venous reflux assessment is simplified (Persson et al, 1986) as demonstrated in figures 1.4.3.4, 1.4.3.5, 1.4.3.6 and 1.4.3.7. Several vessels may be assessed at once for reflux with such a machine. The accuracy of colour flow-mapped ultrasound in defining venous function remains to be proved. Precise anatomical data are obtained, but little numerical information regarding overall venous function can be derived. The information is much more comprehensive, and presumably closer to real events, than that obtained in descending phlebography studies where contrast medium must be injected. The ultrasonic machines described above have the advantage that it is not unpleasant for the patient and can be repeated frequently if necessary since there seems to be no hazard to biological systems from ultrasound energy at the levels used in such machines. It avoids the use of ionising radiation and the expense of radiology suites permitting real-time screening.

Transducers

Colour flow images are produced most commonly by linear sequenced arrays and phased sector arrays although, less commonly, annular arrays are also utilised.

A linear sequenced array is a line of rectangular transducer elements that are operated in groups (sequenced). Each group is energised by a voltage from the pulser in the instrument thus producing an ultrasound pulse that travels through the tissues generating a series of echoes that are presented as a scan line on the display. Subsequent pulses use

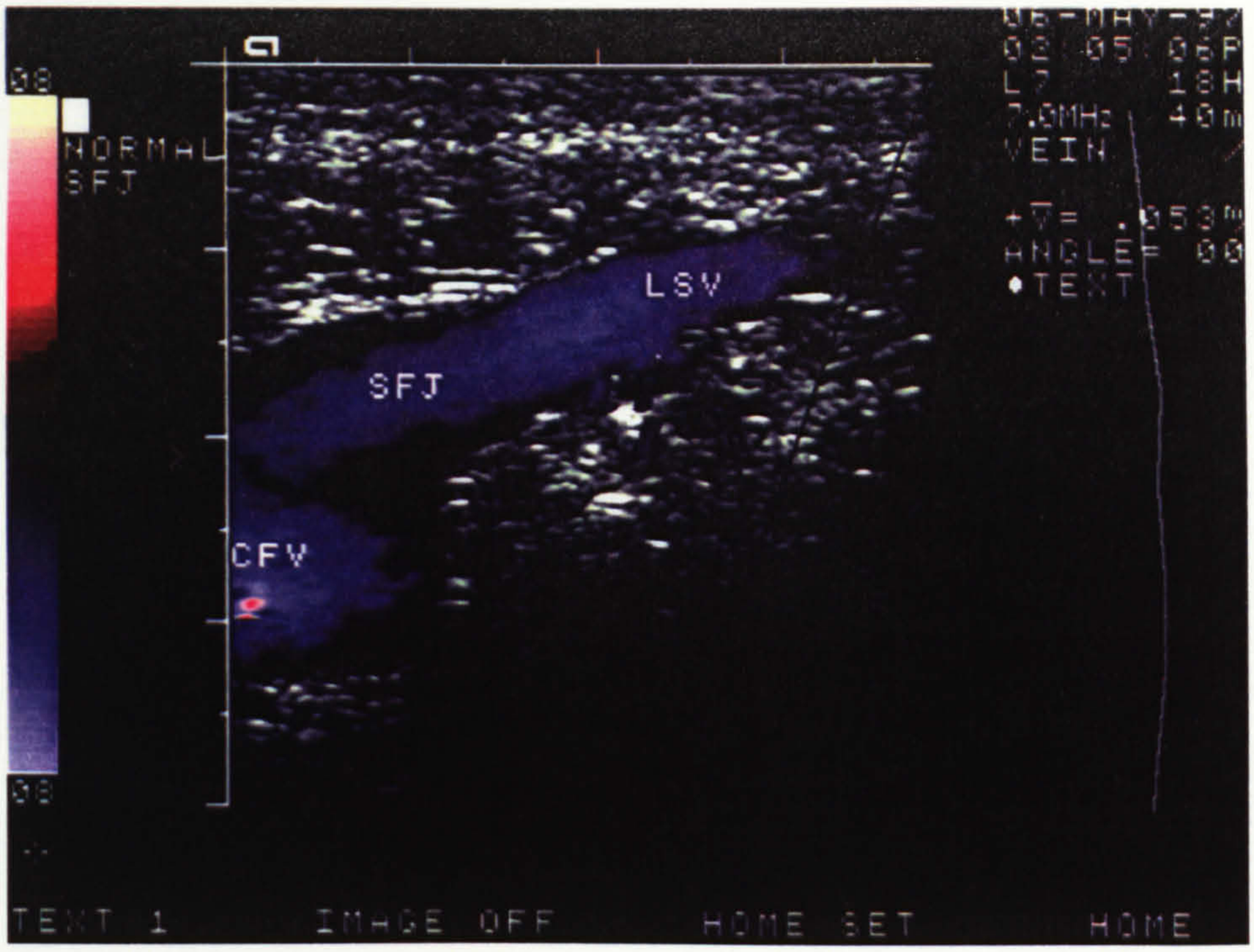

Figure 1.4.3.4 Colour duplex ultrasound image showing a normal common femoral vein (CFV), normal sapheno-femoral junction (SFJ) and a normal long saphenous vein (LSV) depicted by blue pixels indicating normal antegrade flow.

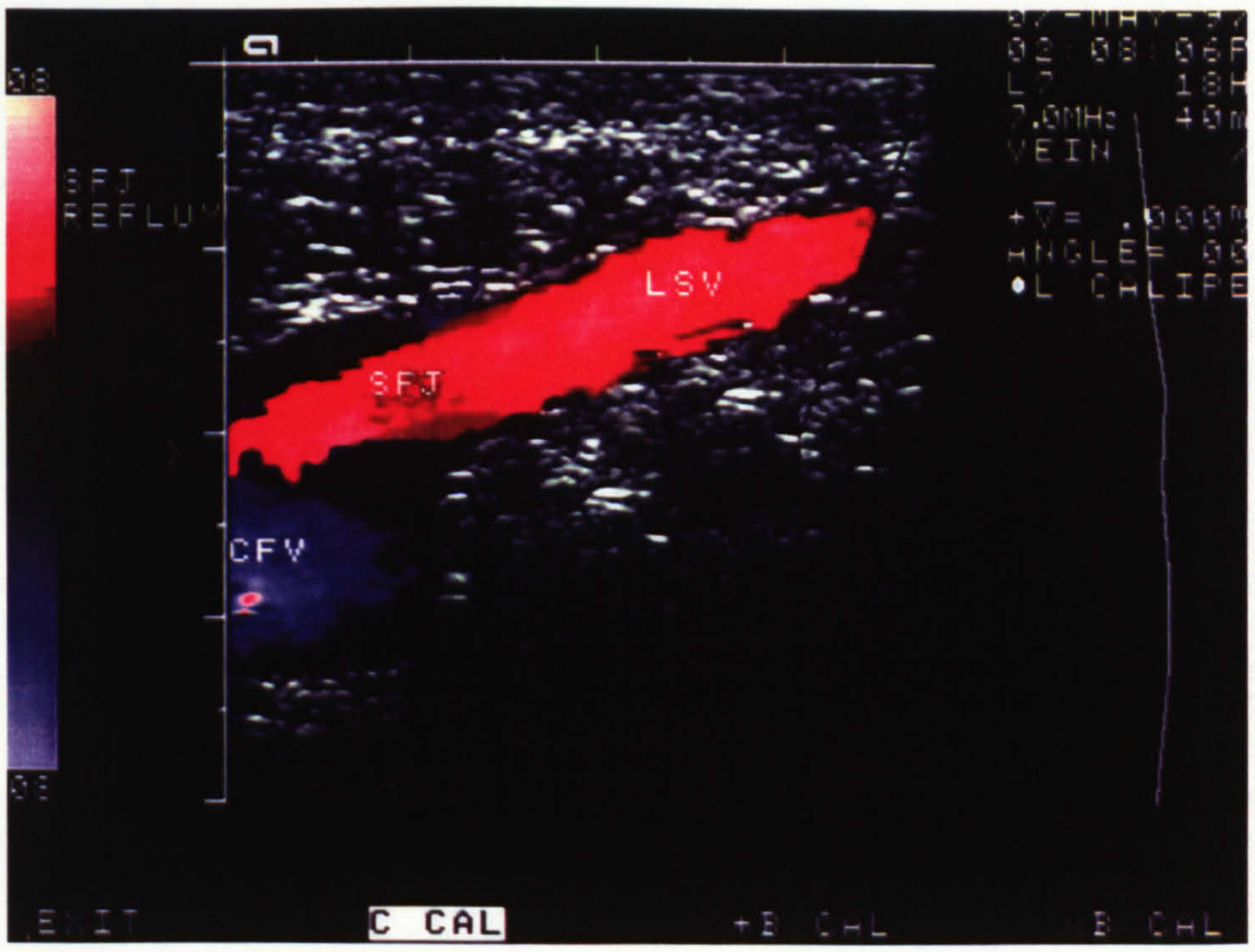

Figure 1.4.3.5 Colour duplex ultrasound image showing a normal common femoral vein (CFV) (blue pixels; antegrade flow), an incompetent sapheno-femoral junction (SFJ) and an incompetent long saphenous vein (LSV) depicted by red pixels indicating retrograde flow or reflux.

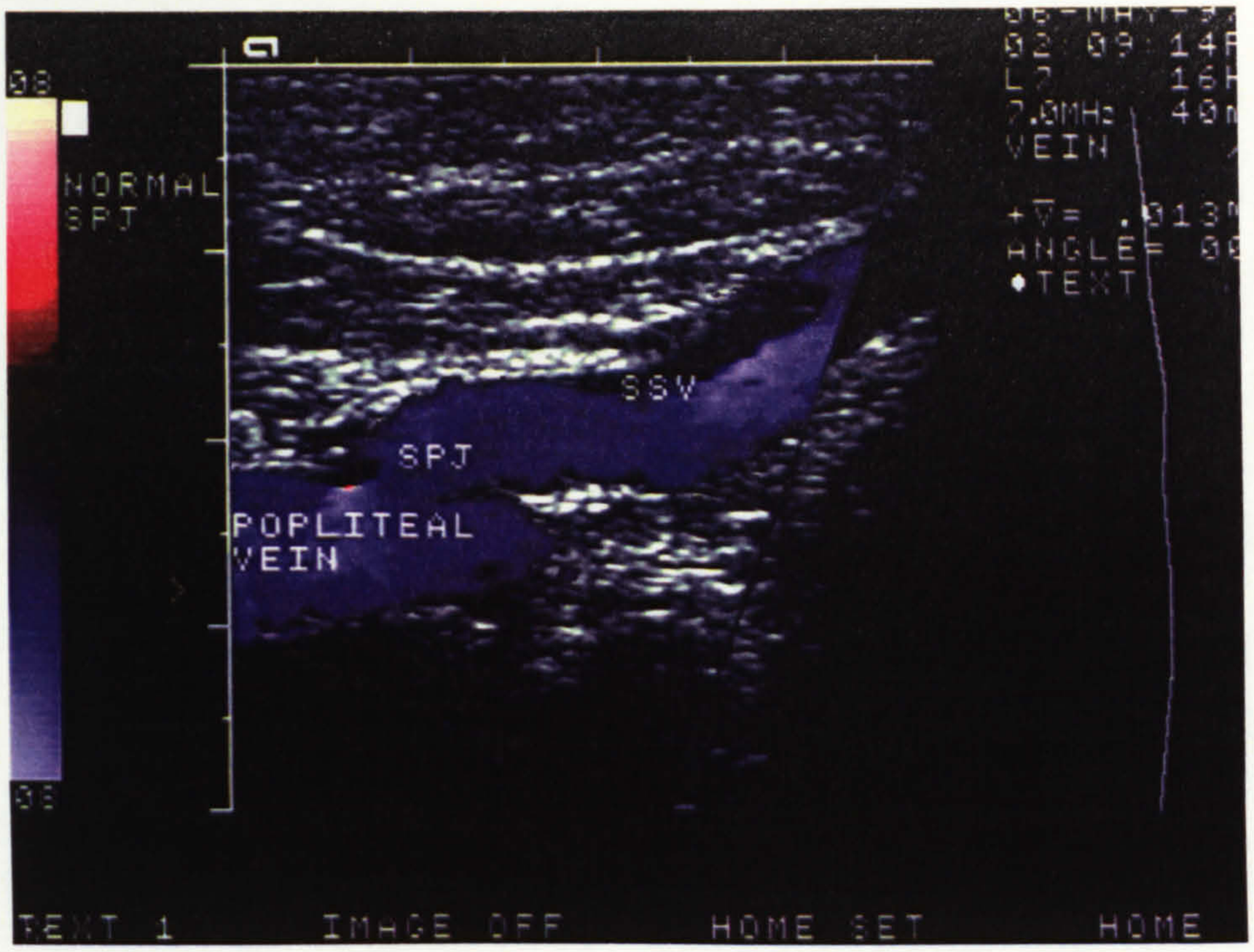

Figure 1.4.3.6 Colour duplex ultrasound image showing a normal popliteal vein, normal sapheno-popliteal junction (SPJ) and a normal short saphenous vein (SSV) depicted by blue pixels indicating normal antegrade flow.

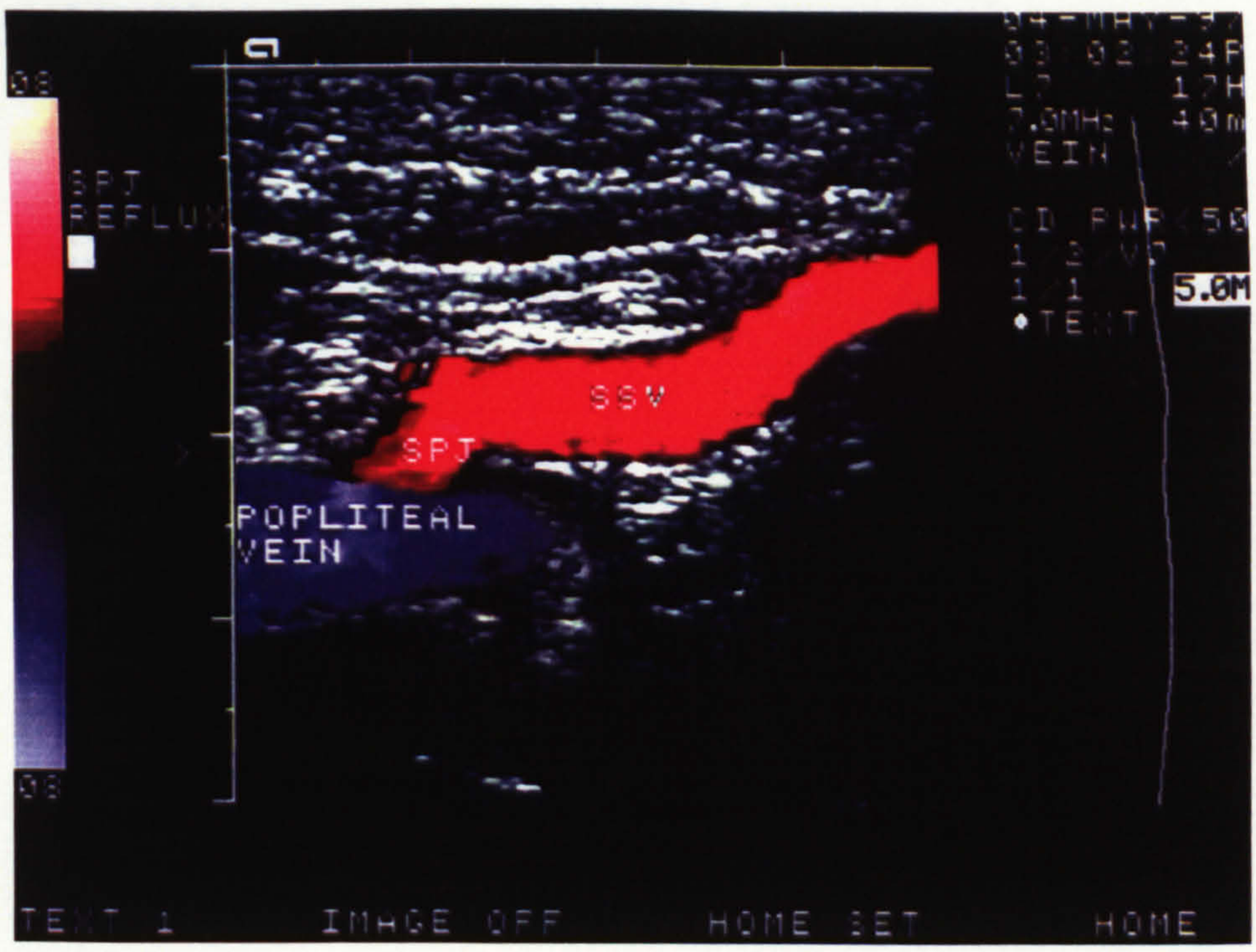

Figure 1.4.3.7 Colour duplex ultrasound image showing a normal popliteal vein (blue pixels; antegrade flow), an incompetent sapheno-popliteal junction (SPJ) and an incompetent short saphenous vein (SSV) depicted by red pixels indicating retrograde flow or reflux.

different groups of elements shifting across the array usually by one element for each subsequent pulse. In a traditional linear array, all pulses travel out in the same direction producing parallel scan lines on a rectangular display.

A phased array is a small line of rectangular elements that are energised in such a way that the direction of the emitted pulse is determined by phasing or delays in the voltage applied to each element. With larger delays the pulses are steered further from the centre. With no delay the pulse travels out perpendicularly from the face of the array. Electronics in the instrument automatically adjust the delays so that each pulse travels out in a slightly different direction from the previous one. This results in an electronically steered sector image.

The transducers described above are therefore used to acquire the grey-scale and colour flow information. The Doppler colour flow instruments are pulsed-Doppler instruments and are subject to the same limitations some of which are described in section 1.4.5.

1.4.4 Cutaneous ultrasound - measurement of skin and subcutaneous layer thickness.

The human skin is a large, complex multi-layered organ with an area of approximately $1.75m^2$ and is the largest organ in the body (Perednia et al, 1995 book). Currently, the most practical modality for imaging the skin is ultrasound. Other methods include histology and electron microscopy.

Ultrasound examination of the skin itself is best performed with 20-MHz transducers (Fornage et al, 1993). However there are limitations to this type of transducer which include the narrow field of view, less than 2cm in width, and inability to visualise the subcutaneous tissues beyond a depth of approximately 1.5cm. These limitations emphasise the need for lower frequencies (7.5 or 10MHz) transducers to image structures that lie beyond the scope of the 20MHz transducers and also because of their low cost and wider availability.

Linear-array transducers are preferred to mechanical sector probes of the same frequency because of their better near-field resolution and wider field of view. Also, the ultrasound beam of the linear-array transducers is perpendicular to the skin surface and most of the underlying interfaces in the subcutis. This is important because it minimises the scattering artefacts. The ACUSON 128 XP/10v (Acuson, Mountain view, California) 7.5MHz linear-array probe has a field of view of about 4 cm wide and 4 to 6 cm deep.

Brightness mode or B-mode ultrasound can be applied to study the skin in different ways. The ultrasound collects the data and presents it as a digitised two-dimensional picture with bright pixels representing strong reflections and dark pixels depicting the absence of reflections. As the transducer is moved across the surface of the skin with coupling gel applied, ultrasound pulses are transmitted and echoes received from each transition zone where the acoustical properties of the tissues differ. The returning echoes are timed and the returning times multiplied by the speed of sound in the tissues to determine the distance travelled before reflection. The strength of the echo at each point is converted to a brightness value between 0 and 255, with the stronger echoes given a higher brightness. When all the individual readings are placed beside one another over the scan length of the transducer, a two-dimensional B scan image is formed. B-mode displays represent a pictorial cross-section of the skin and subcutaneous tissues in a visual way.

Skin contains collagen which is an important source of echogenicity in any tissue. Cells and collagen fibres intertwined within the dermis produce a bright or 'echogenic' layer within the B-scan map. The subcutaneous fat is a homogeneous layer which contains little collagen with relatively few areas in which tissue structure and therefore sound impedance changes, resulting in dark or 'echolucent' structures on the B-scan image.

In normal skin the interface between the dermis and the subcutaneous fat is far from smooth due to attachments of subcutaneous retinacula. The anatomical thickness of the subcutaneous fatty layer is even more variable. A precise distance measurement can never be attained due to the biology itself being so 'noisy' even if the ideal scanner existed.

Normal Ultrasound Anatomy.

Using a 7.5MHz linear-array probe, the skin appears as a thin, regular layer of tissue that is more echogenic and sharply demarcated from the underlying hypoechoic subcutaneous fat (Fornage et al, 1986; Miyauchi et al, 1983).

The interface between the echogenic skin and the hypoechoic subcutis is clearly depicted in most areas of the body, allowing measurement of the skin thickness, although with less accuracy than at higher frequencies (Fornage et al, 1993; Fornage et al, 1986).

The subcutaneous tissues are composed of hypoechoic fat with a few scattered echogenic strands of connective tissue.

Ultrasound has also been used to measure the thickness of subcutaneous fat at various sites in normal subjects (Maruyama et al, 1991).

Ultrasound is widely used to evaluate changes in the thickness of subcutaneous fat associated with various clinical conditions in humans (Gooding et al, 1986; Heckmatt et al, 1988).

The use of 7.5- and 10-MHz transducers are ideal for use in the ultrasound imaging of the subcutaneous tissues with the added advantages of being low cost, versatile, quicker to perform and more widely available than any other cross-sectional imaging modality currently available. When recording B-scan images for analysis, one should consider that the received ultrasound signal is influenced by attenuation due to absorption and/or scattering and reflection. Attenuation is the decrease in amplitude and intensity as a wave travels through a medium. Reflection takes place mainly at the boundaries of tissue compartments. Absorption is high when the relative amounts of proteins and collagen in the tissue increases and low if the liquid content is elevated. As a result of attenuation, a signal coming from a given tissue structure varies not only to the acoustic tissue parameters but also to the distance between the structure and the transducer. This is why it is very important to keep the probe-to-skin distance constant by using a gel stand-off pad between the probe and the skin as this improves the examination of the skin and superficial subcutaneous tissues with the skin being closer to the focal zone (Fornage et al, 1984).

While the ultrasound examination of the skin is possible at these frequencies it is best done with transducers of 20 MHz or more. However, the limited penetration of these very high frequency transducers does not allow adequate examination of the subcutaneous tissues (Fornage et al, 1995).

1.4.5 Effect of physiologic and system hardware variables on the accuracy of quantitative volume blood flow measurements using duplex ultrasound.

Time averaged velocity

Inaccuracies in estimating volume blood flow with ultrasound arises in the calculation of several values including the time averaged velocity (TAV), which is the average rate of flow through the vessel over a period of time.

The TAV measurements are calculated by the ACUSON 128 system computer software. The introduction of errors during TAV measurements could arise from contamination due to background noise as a result of poor signal-to-noise ratio, tissue and wall motion and/or signals from vessels other than the target vessel. Solutions to errors arising

during TAV measurements which will improve the accuracy of volume flow calculations includes placing the baseline in the centre of the spectral Doppler display which will result in equal amounts of noise on both sides of the baseline with the mean of the positive and negative noise balances approaching zero. Strong Doppler signals should be obtained which will result in less impact from background noise. The relative amplitude of background noise is small compared to Doppler shifts from moving blood.

Incorrect angle of insonation

Incorrect angle of insonation can also contribute to the possibility of incorrect estimation of volume flow at the point of spectral sampling.

As in all Doppler examinations using velocity information, the angle of the blood flow within the vessel relative to the Doppler beam must be computed. Errors of several degrees can lead to large changes in the cosine value used in the Doppler formula, with resultant miscalculation of the TAV values. If the probe beam is placed perpendicular to the blood flow with a scanning angle of 90 degrees, it would yield no Doppler shift, and it would indicate that there is occlusion or no flow in the vessel under investigation even though the vessel is actually patent. The probe beam should be oriented to make a 30 degree to 60 degree scanning angle with the vessel lumen and angles greater than 60 degrees should be avoided. According to the Doppler equation, the angle between blood flow and the Doppler beam is a critical factor because of the cos θ term. An important point to remember is that what the transducer measures is the $v \cos \theta$ component of blood flow. Slight inaccuracies in estimating an angle less than 60 degrees results in small or insignificant errors in actual flow measurement while the same inaccuracy at an angle greater than 60 degrees will provide extremely large errors.

Aliasing

Aliasing is an artefact that can be noted on the Doppler spectrum. The information contained by the higher frequency shifts is displayed at a lower frequency. The electronics of the ultrasound machine can process the information from the ultrasound echoes at a maximal rate defined by the pulse repetition frequency (PRF). If the PRF increases, higher frequency shifts can be displayed and aliasing is less likely. If the PRF decreases, there is a greater chance of aliasing occurring.

Inaccurate cross-sectional area measurements

Inaccurate cross-sectional area measurements is another important factor in the incorrect estimation of volume flow at the point of spectral sampling. The area of the vessel should be measured at the same point in the vessel that the Doppler sample volume was acquired. Because Doppler samples must be taken using longitudinal views of the vessel to assure quantification accuracy, the only way to use the same image is to measure the diameter of the vessel to calculate the radius and then use that value to calculate the area ($A = \pi r^2$ or $\pi D^2/4$).

Vessels not imaged at 90 degrees can also result in inaccurate measurement of the cross-sectional area. It is easy to be at a slight angle to the vessel rather than perpendicular even with careful technique. An ultrasound slice that is not perpendicular to a vessel will create an elliptical structure. The area of an ellipse is larger than a circle, and will result in an overestimation of cross-sectional area. When using a diameter measurement from a longitudinal view, the beam may not cut through the vessel at exact points on opposite sides of the vessel. The diameter will be less than the true value, introducing error into the value for radius. This error will be further magnified when the radius is squared to calculate area.

1.5 Tissue tonometry

At present, there is no standardised objective method of assessing the degree of skin changes seen in patients with lower limb chronic venous disease. A tissue tonometer has been developed in our laboratory for the objective assessment and quantification of the skin changes seen in these patients.

1.5.1 Previous models

An instrument which measured the compression of tissues by a mass was developed in the seventies to objectively determine the pitting in lymphoedema (Clodius et al, 1976). This instrument, known as the Clodius tonometer, consisted of a plunger connected to an analogue scale. Weights were added to the plunger and the distance travelled by the plunger was measured. Several studies using this instrument to quantify the pitting in oedema have been published (Piller and Clodius, 1976; Chen et al, 1988; Kar et al, 1992). However, the Clodius instrument had two major drawbacks. Firstly, the instrument was top heavy making it unstable and secondly, the instrument measured only the depth of compression and gave no indication of the rate of compression and the depth of pitting measured was time dependent. The problem of stability was addressed by Chen et al in 1988 to fix the instrument with a moveable grip.

A durometer has been used to determine the degree of induration in LDS by Romanelli and Falanga (Romanelli et al, 1995) based on previous work done on the assessment of skin hardness in patients with systemic sclerosis (scleroderma) by Falanga and Bucalo (Falanga et al, 1993). The durometer is the international standard for measurement of hardness of plastic, rubber and other non-metallic materials, and it has a calibrated gauge that linearly registers the relative degree of hardness. This instrument senses hardness with a spring loaded indentor (plunger) and assesses the distance travelled by the plunger into the material under test. The durometer judges only one aspect of the material or tissue to which it is applied, that is, its elasticity. The skin and subcutaneous tissues is a complex structure and we suspected that it would be useful to assess a number of other mechanical properties of this to provide a complete picture of the changes produced by venous disease.

An instrument to objectively record the depth and rate of pitting in lymphoedematous arms was developed by Bates et al in London (Bates et al, 1994) based on the Clodius tonometer. It consisted of a plunger attached to a lever which deflected a strain gauge transducer. The deflection caused a change in voltage output of the transducer, which

was amplified and recorded on a chart recorder. The pitting curves obtained were analysed in terms of distance and rate constant parameters. A mathematical model (see section 1.5.3), the Kelvin-Hooke model was proposed to analyse the pitting curves obtained from the instrument and to determine the spring and dashpots constants.

1.5.2 The Middlesex Hospital Vascular Laboratory tissue tonometer

A computerised tissue tonometer was developed at our laboratory (The Middlesex Hospital Vascular laboratory, 1995) and is based on a model designed by Bates et al described above. It is a simple, non-invasive device which consists of a plunger which may be loaded with different weights (figures 1.5.1, 1.5.3, 1.5.4. 1.5.5 and 1.5.6). The plunger is attached to a cantilever bar which is on a pivot and connected to a linear variable differential transformer (LVDT) position detector, a sensing device, that detects the movements of the plunger which is positioned on the skin. The plunger can be released by a cable release mechanism and is allowed to sink into the tissues under gravitational force. The signals obtained by the LVDT in the tonometer are converted by an analogue to digital (A/D) converter in the computer into the traces displayed on the computer screen. Though our model is based on the Bates's tonometer, I have modified certain aspects of my tonometer to suit our measurements. I have installed an electronic sensor within the tonometer connected to an analogue to digital converter in an IBM compatible computer to analyse the traces electronically on a computer screen. During measurements, I used a weight of 30g and a plunger size of 4mm diameter in all the studies so as to standardise the measurements. The weighted plunger was allowed to sink into the tissues following release.

Typical traces obtained from the computer consisted of an initial fast indentation phase followed by a subsequent slower indentation phase or creep phase (figure 1.5.2).

The traces obtained were analysed using specially written computer software (by PDCS) to calculate the distance and rate constant parameters. The traces were divided into three phases: phase I, phase II and phase III.

Phase I occurs within the first second to reflect the initial rapid indentation phase. The start cursor is placed at the site indicating where the plunger is released unto the tissues and the end cursor is placed at the end of the straight line just before the start of the creep phase as shown in figure 1.5.2. The distance between the start and end cursors will give the distance travelled in mm during phase I.

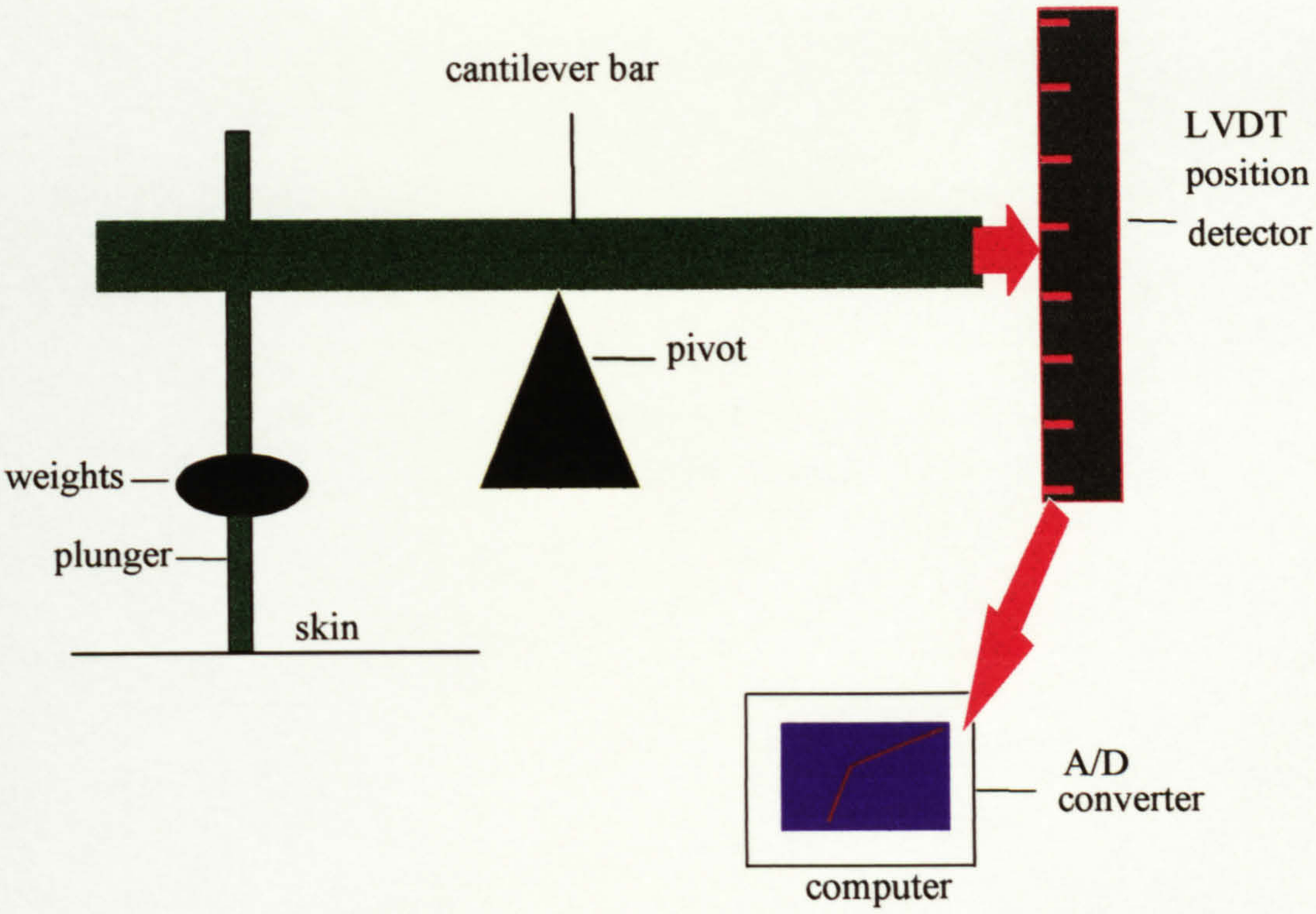

Figure 1.5.1 **Schematic diagram of the tissue tonometer showing the plunger with weights which is attached to a cantilever bar on a pivot that is connected to a sensing device that detects the movements of the plunger on the skin and sends signals to a computer via an analogue to digital converter to convert the signals into traces.**

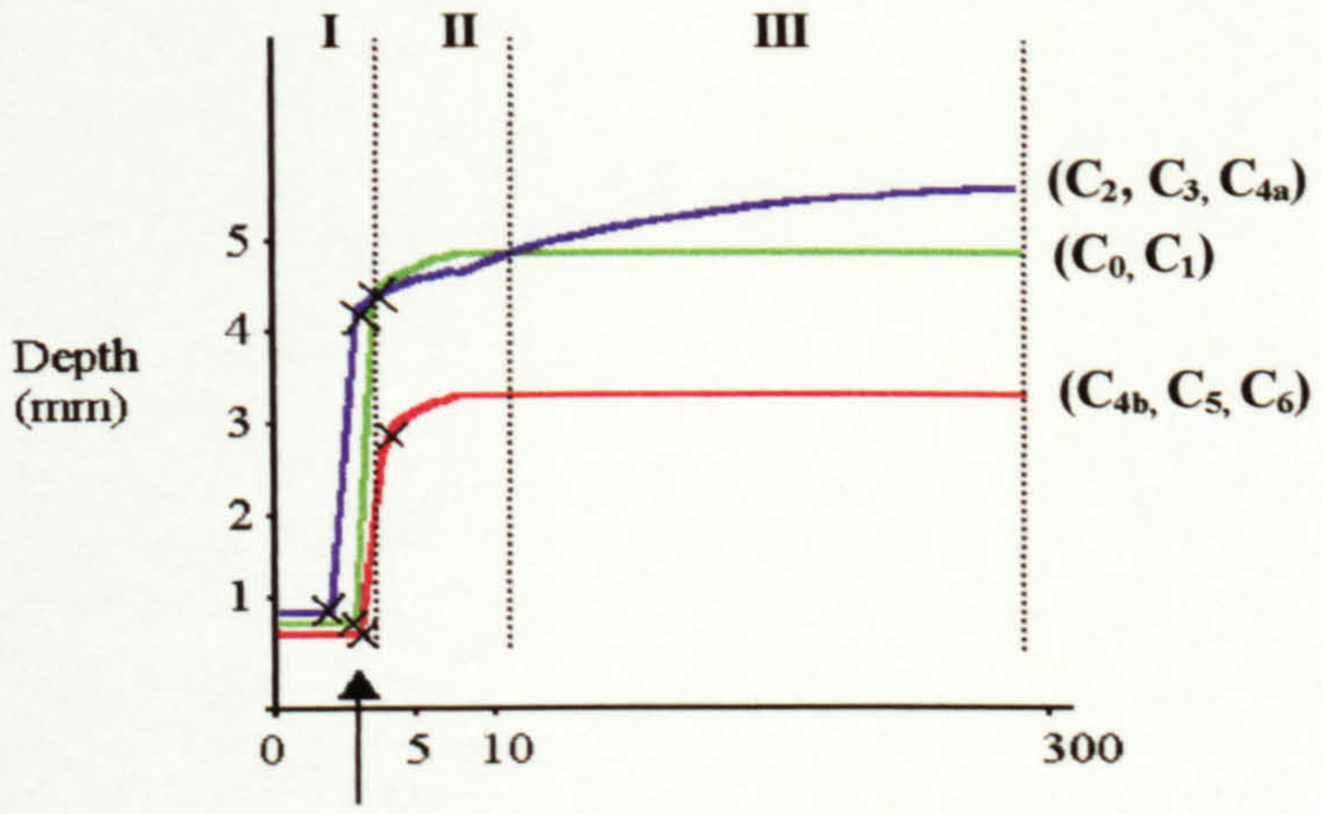

Figure 1.5.2 **Typical traces obtained from displacement versus time curves using a tissue tonometer. The arrow indicates the time the plunger is allowed to sink into the tissues. The initial fast indentation phase, phase I, which occurs within 1 second, is indicated by the black crosses and is followed by the subsequent slower indentation phase which has been divided into phase II (1–10 seconds) and phase III (10 – 300 seconds).**

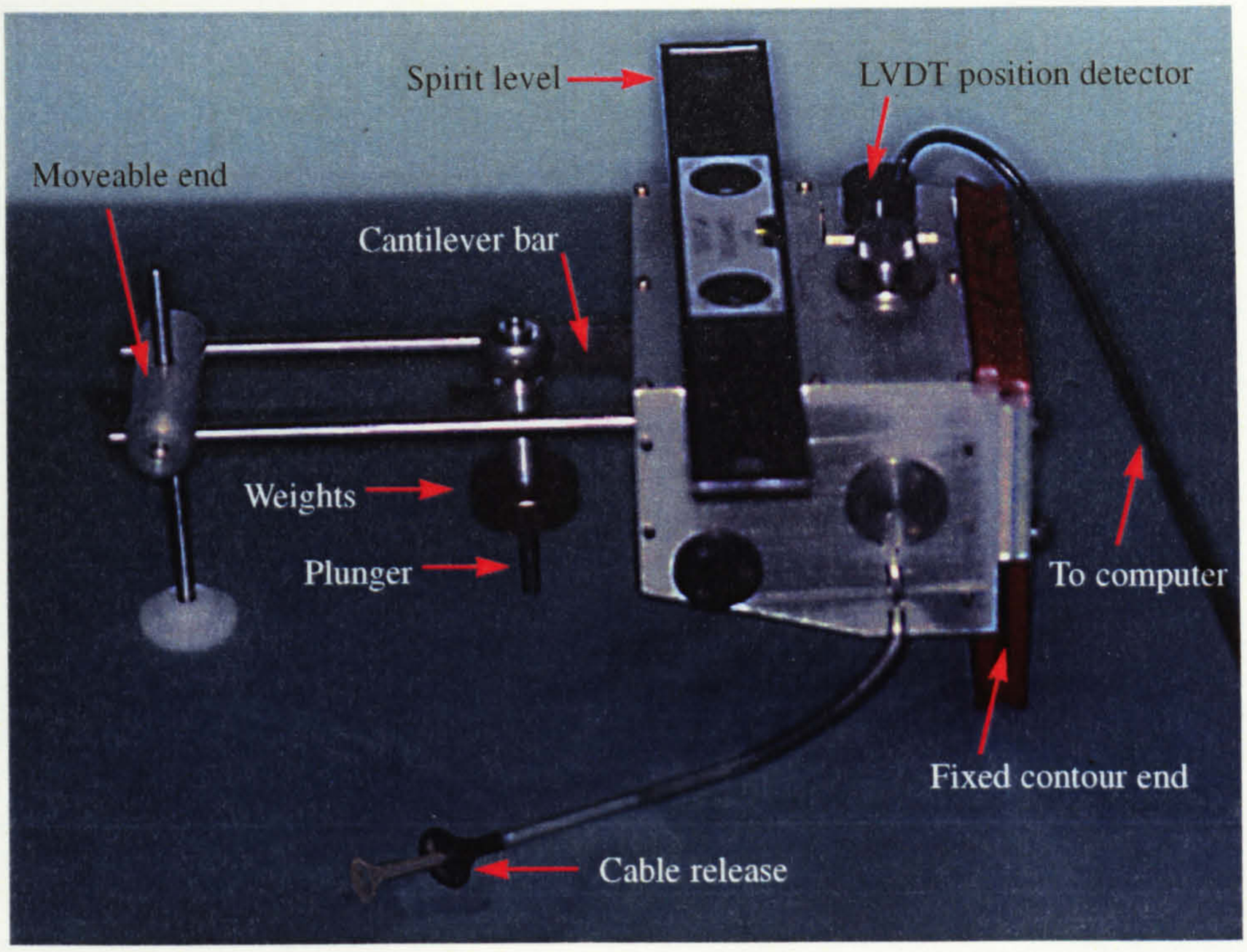

Figure 1.5.3 The tissue tonometer showing the fixed and moveable ends with the plunger and added weights attached to a cantilever bar which is connected to a linear variable differential transformer (LVDT) position detector that sends signals to an analogue/digital converter present in a computer. The spirit level is used to ensure that the cantilever bar is parallel to the surface of the table during calibration.

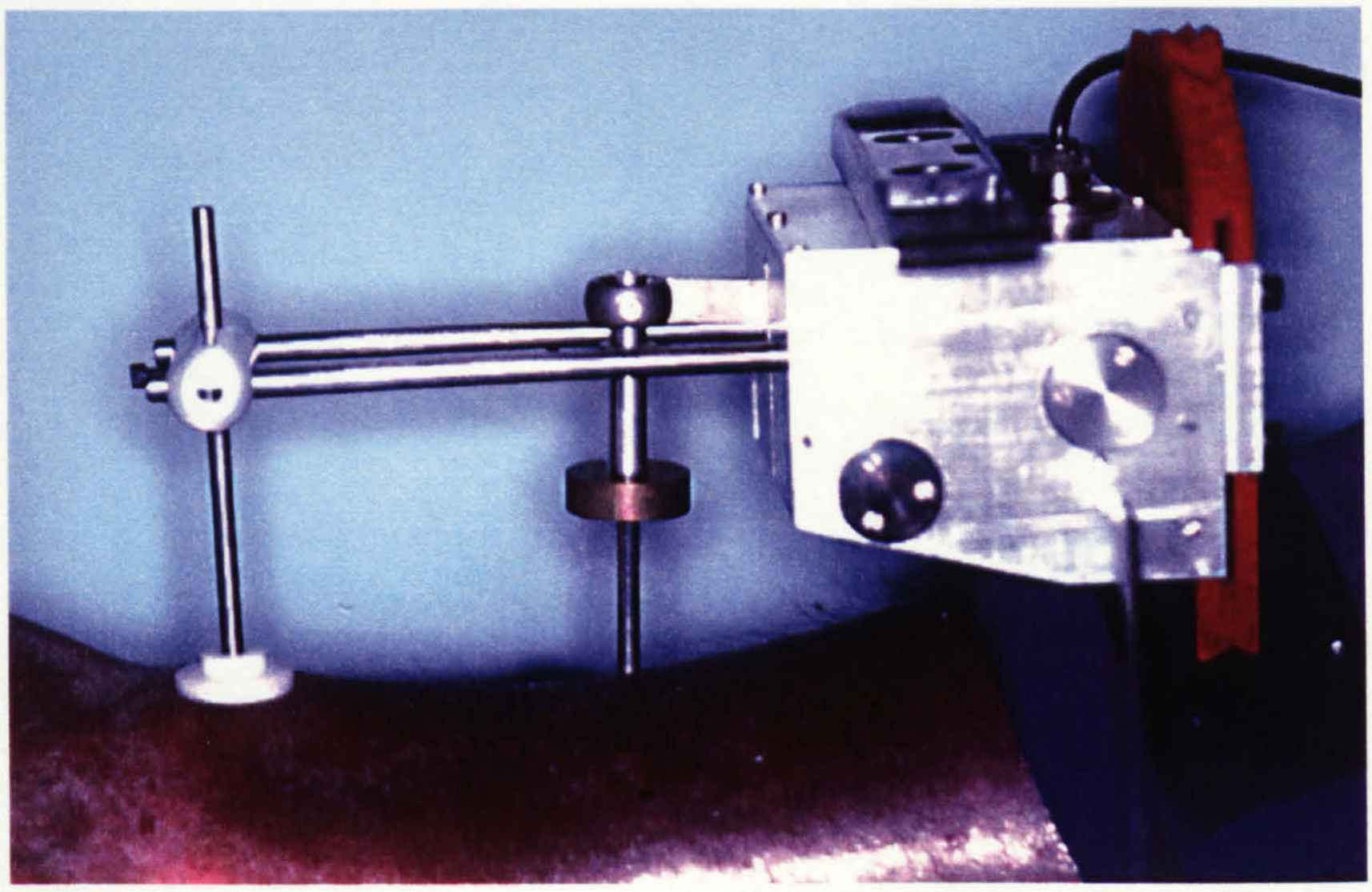

Figure 1.5.4 After calibration, the tonometer is placed on the subject's limb with the plunger positioned over the area of interest, the gaiter area, of patients with lower limb chronic venous disease.

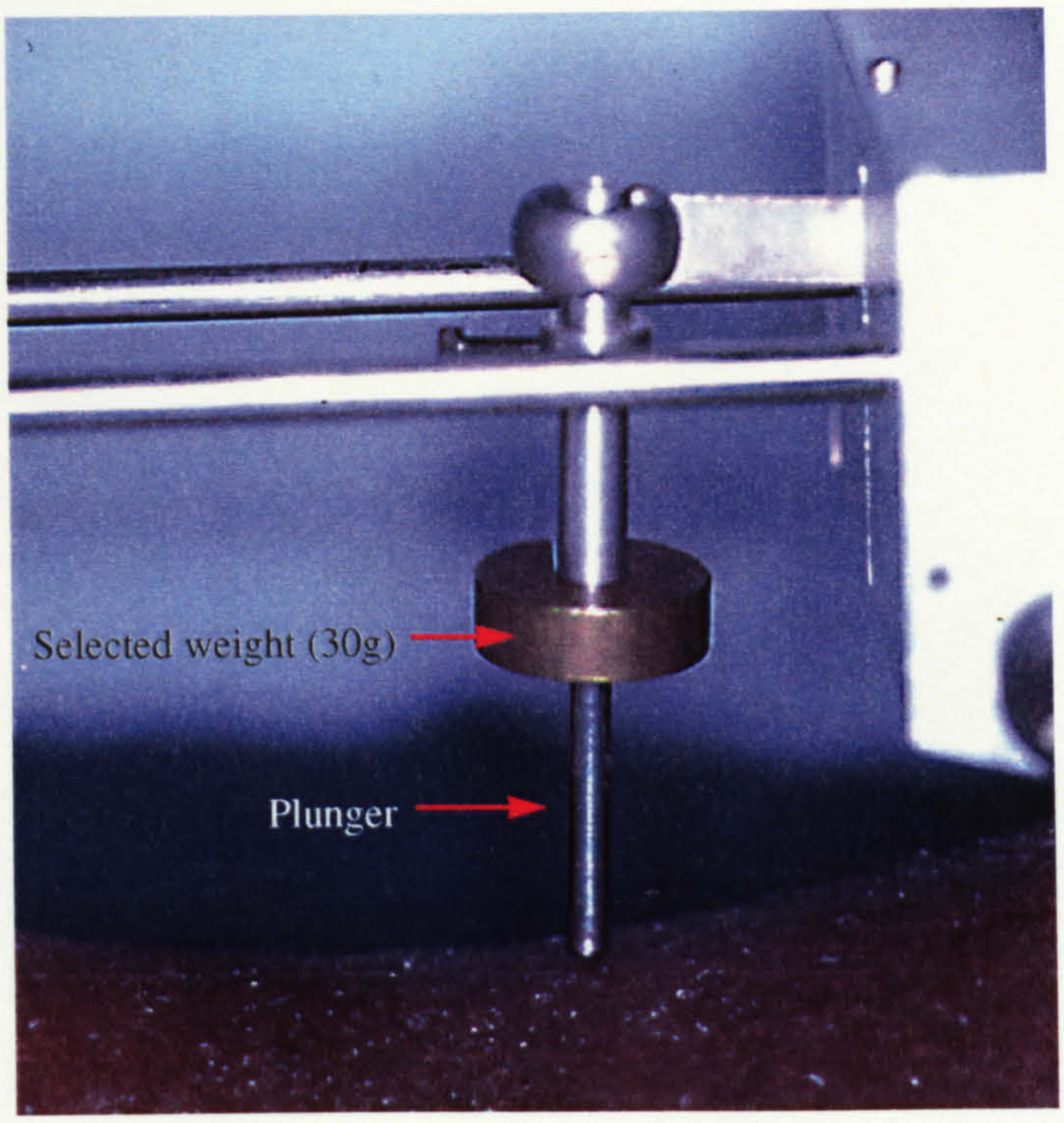

Figure 1.5.5 The plunger with the selected weight is positioned just above the area of interest without causing indentation.

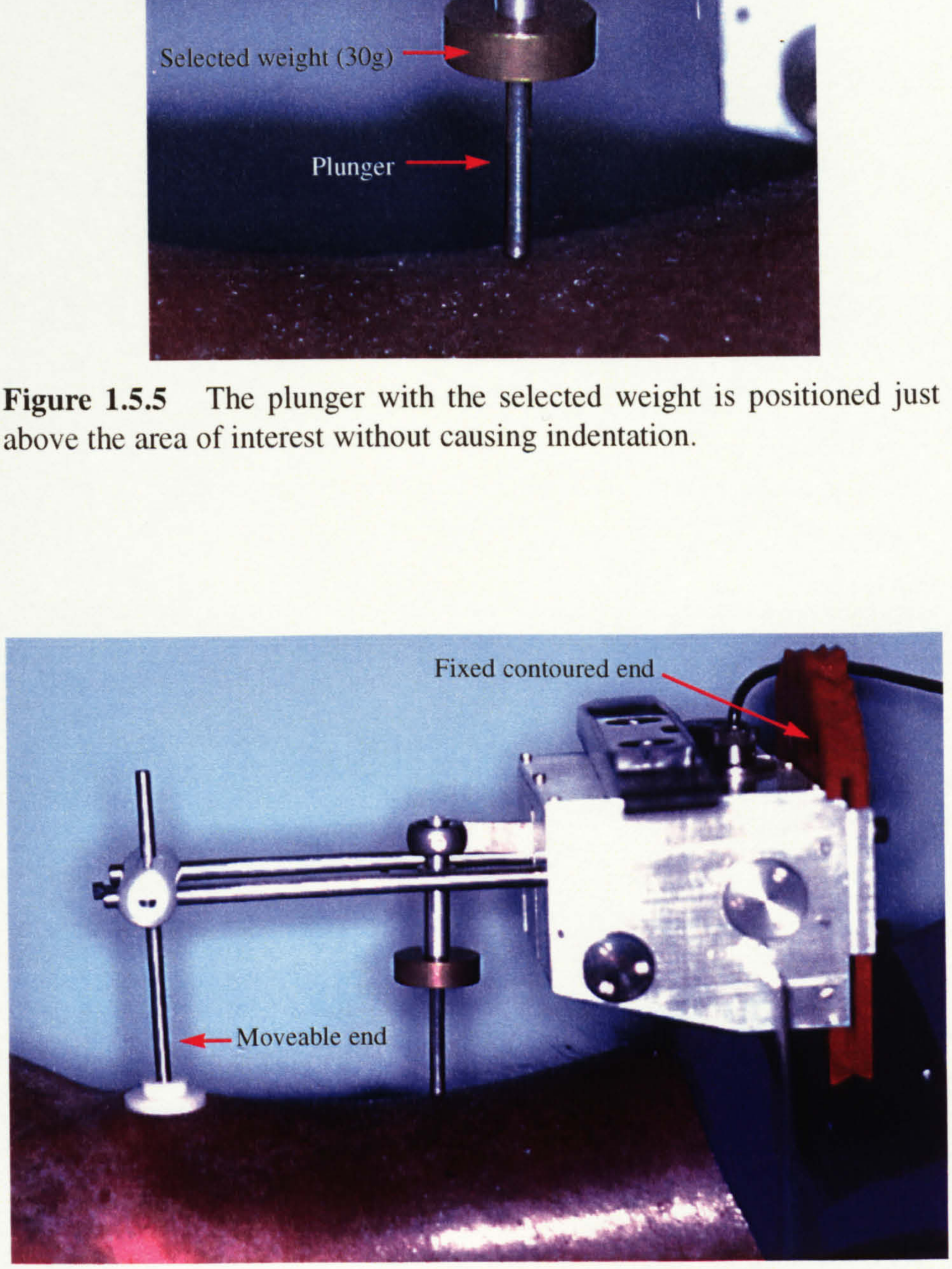

Figure 1.5.6 The tonometer is placed on the subject's limb with one end contoured around the calf (red-end) and resting on velcro material to prevent slipping. The other moveable end can be moved along the bar to any desired site to ensure maximum stability.

I thought it was necessary to subdivide the subsequent slower indentation phase or creep phase into phases II and III with the former lasting from 1 to 10 seconds and the latter lasting from 10 to 300 seconds because a mono-exponential regression line did not fit the whole of this part of the trace and it was better to use two exponential regression lines. For phase II, the start cursor is placed at the beginning of the creep phase and the end cursor is placed at the 10 second mark which is shown on the computer screen. The specially written software then plots a mono-exponential regression line to provide the distance parameter in mm and the rate constant parameter (-1/tau in seconds^{-1}) which is the slope of the line. For phase III, the distance and rate constant parameters are calculated from a mono-exponential regression line after the start and end cursors are placed at the 10 second mark and 300 second mark respectively.

The tissue tonometer had been adapted to cope with the change in speed between the initial fast indentation phase and the subsequent slower indentation phase with the recording mode in the first ten seconds of indentation, the rapid indentation phase, set at 40 samples per second which automatically switched to a slower speed of 4 samples per second to record the relatively slower subsequent indentation phase in the last 290 seconds.

Five minutes of recording following release of the plunger was selected for all measurements using the tissue tonometer after it was observed in preliminary measurements that the trace levelled off before 5 minutes had elapsed and that five minutes recording was reasonable before patient fatigue and/or restlessness sets in which may introduce 'noise' into the data.

1.5.3 Analysis of displacement versus time curves obtained with tissue tonometry

The Kelvin-Hooke model

The displacements versus time curves obtained from the tissue tonometer both *in vitro* (compression of glycerol soaked sponges) and *in vivo* (normal control subjects and subjects with evidence of lower limb chronic venous disease), showed an initial rapid indentation phase followed by a subsequent slower indentation phase or creep phase.

In this thesis, I have applied a simple mathematical model, the Kelvin-Hooke model, to explain the tonometry curves obtained for all *in vitro* and *in vivo* studies in measuring the mechanical properties of the skin in lower limb chronic venous disease. This model was previously used by Bates et al in 1994 and was shown to fit his tonometry results in post-mastectomy lymphoedematous arms. Since the skin and glycerol soaked sponges show visco-elastic properties, this simple mechanical model generates curves of the type described above and would be used to explain the curves obtained with the tissue tonometer. It is a simple mathematical spring and dashpot visco-elastic model, which has a Kelvin and a Hooke element in series (Viidik et al, 1973) and is explained in details below.

A force is required to alter a specimen of material by mechanical means. Rheology is the study of the properties of a material which are time-dependent. Macrorheology is the part of rheology that is confined to the study of the external property changes due to external forces as in the deformation of the specimen. Many of the changes that occur in a material can be described by certain basic elements that can be combined to provide rheological models. These models will help to visualise the mechanical properties and to facilitate their mathematical description but will not necessarily correspond to the physical events within real materials.

Elasticity is symbolised by a perfect spring which is extensible without limits and with a constant spring rate (c) and is also known as the Hooke element which is represented by a spring attached to a wall (figure 1.5.7) and obeys the equation

$$\mathbf{F = cX,}$$

Where X is the deformation and F is the load.

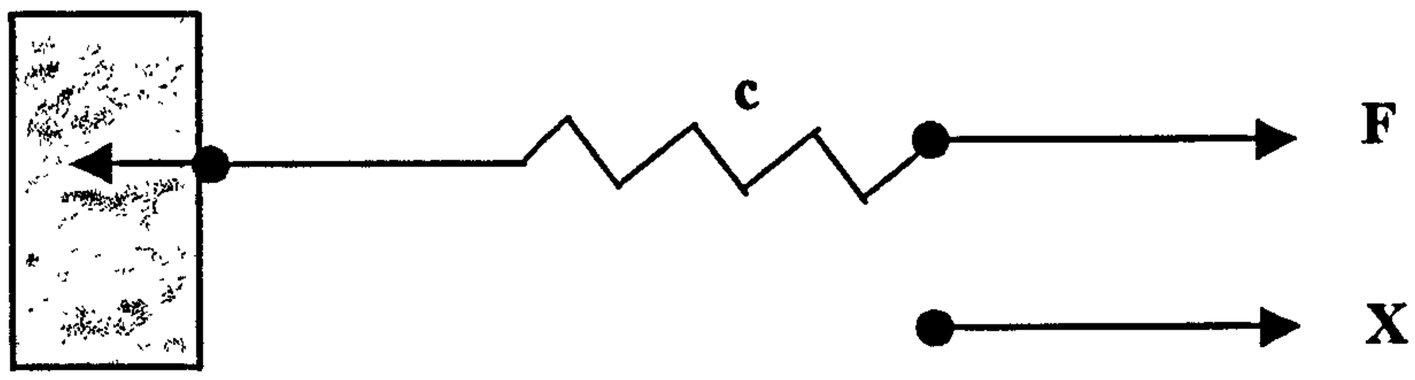

Figure 1.5.7 The Hooke element of an ideal spring attached at the left to a fixed point and at the right, the load (F) is applied and the deformation (X) is measured.

All loadings, independent of speed, follow the same curve (i.e. a straight line) and are completely reversible along the same curve (Figure 1.5.8) This means that no energy is dissipated and there is no time dependence with all responses being instant.

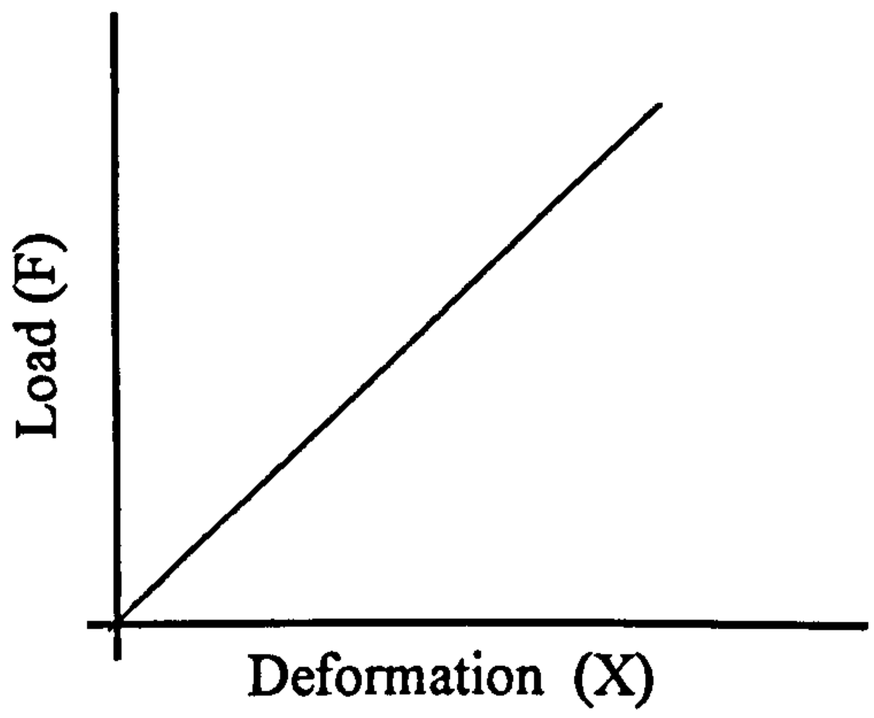

Figure 1.5.8 The load-deformation curve for the Hooke element of ideal or linear elasticity.

Viscosity, represented by the Newton element, is visualised by a viscous damper or dashpot (Figure 1.5.9). The dashpot functions as a syringe with a small gauge needle i.e., the more fluid to be injected per unit time, the more pressure needed on the syringe plunger (note that in the mathematical model the flow is between the cylinder wall and the piston whereas in the syringe it is through the needle). On the other hand, the more 'viscous' is the fluid, the more pressure is required on the plunger to inject the same amount of fluid per unit time. This is expressed mathematically

$$\mathbf{F} = k\,\dot{X}$$

Where k is the damping constant and $\dot{X}$ is the velocity or time derivative of deformation which can also be written as dX/dt. Therefore the above equation becomes

$$F = k \cdot dX/dt$$

If the load and velocity are constant, the time-deformation relationship is a straight line as shown in figure 1.5.10. This means that if the velocity and/or coefficient of viscosity is very low, and $\mathbb{X} \cong 0$, then almost no counterforce is created in the dashpot.

On the other hand, if a sudden load is applied, this will result in the 'speed' becoming very high and $\mathbb{X} \rightarrow \infty$, the dashpot would become locked and behave initially like a rigid body with very little fluid flowing through the space between the piston and the cylinder with the pressure in the fluid becoming very high.

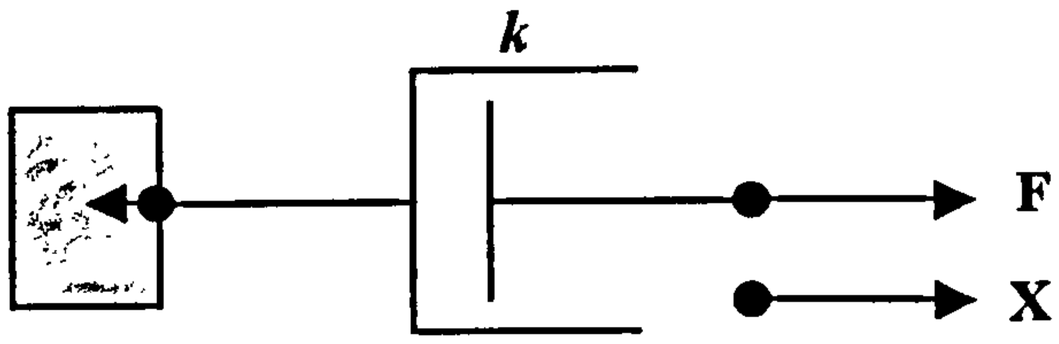

Figure 1.5.9 The dashpot, *k*, symbolising the Newton element of linear viscosity.

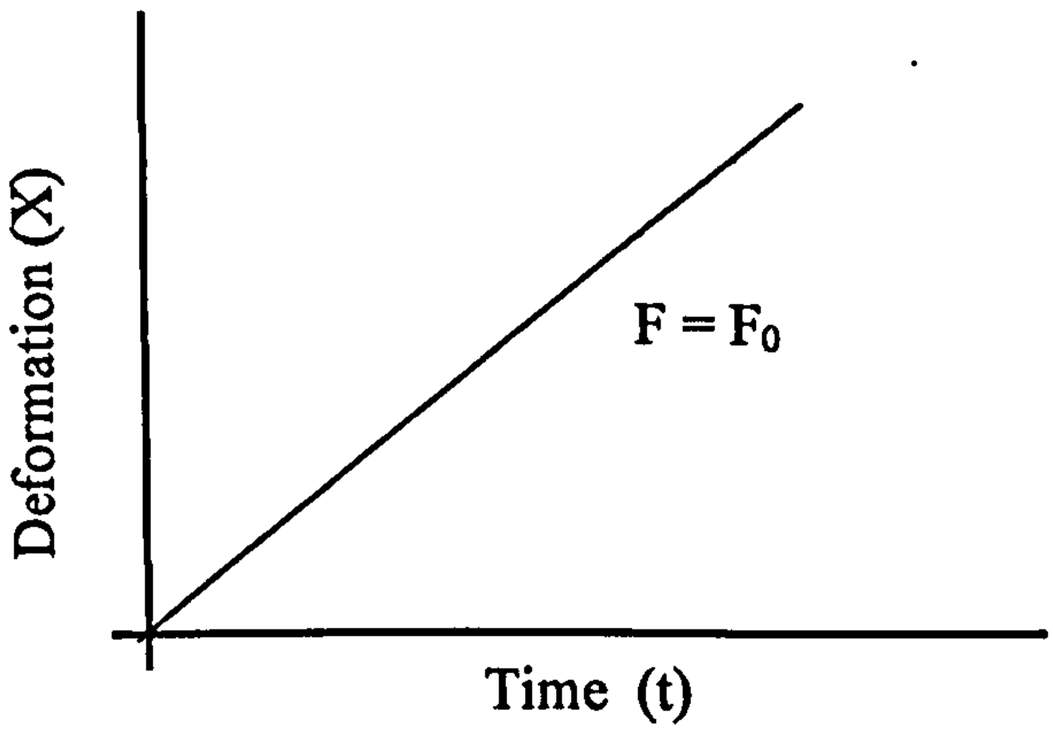

Figure 1.5.10 The deformation-time relation for the Newton element of linear viscosity when the constant load of F_0 is applied to it.

The most simple combinations of the idealised elements are those containing an elastic and a viscous element in series or parallel. When the elements are coupled in parallel, the system is known as the Kelvin element shown below in figure 1.5.11

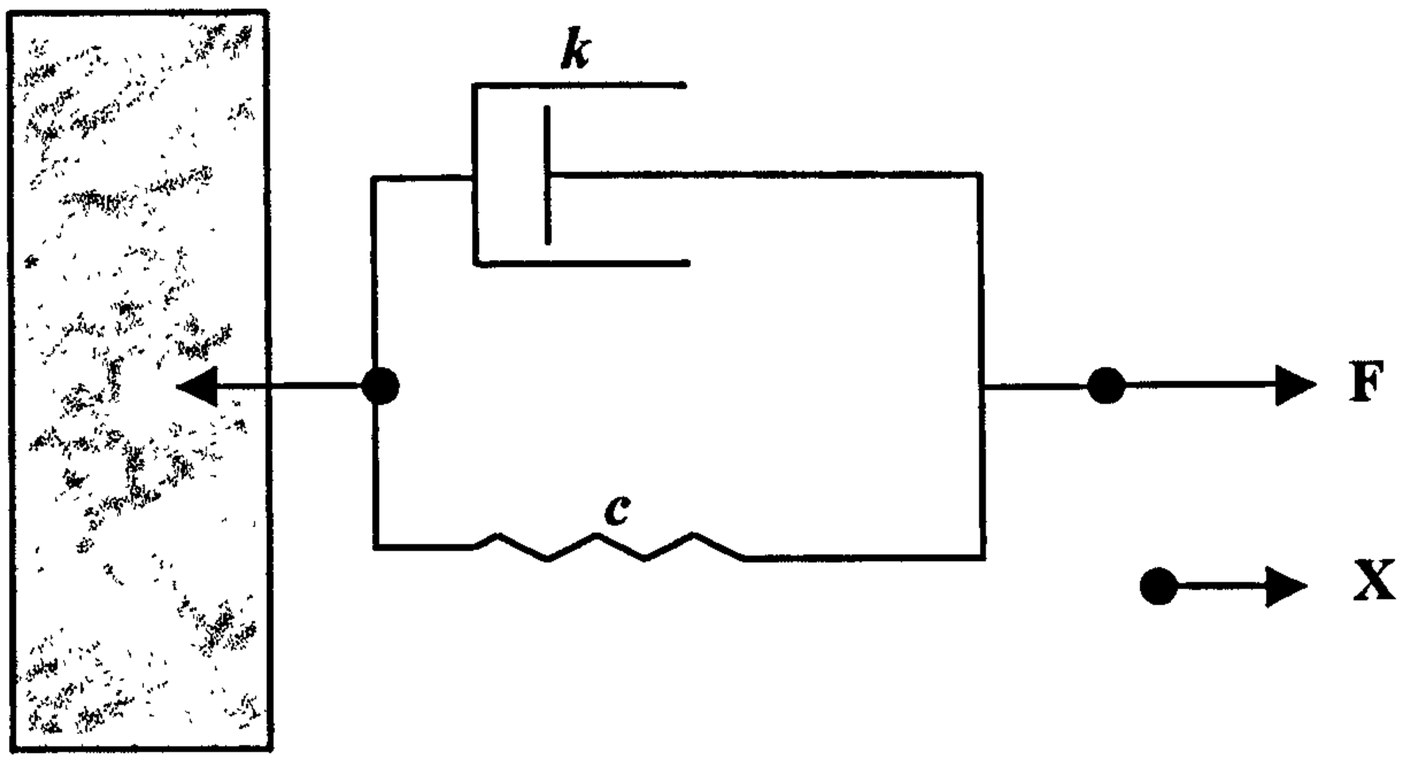

Figure 1.5.11 The Kelvin element is the Hooke and Newton elements coupled in parallel.

In this system, both elements are always subjected to equal deformation while the total load is distributed between the two, thus,

$$X_c = X_k = X$$

$$F_c = F_k = F,$$

which gives the equation for the system

$$F = cX + k\,X$$

And if the load F0 is applied at time t = 0 and X = 0, a gradually increasing deformation occurs

$$X = F_0 [\, 1 - \exp (- ct / k) \,] / c$$

The above equation suggests that initially most of the counterload is generated in the dashpot and with increasing deformation the counterload is gradually transferred to the spring as the system is elongated. The load in the dashpot is exhausted asymptotically and at $t = \infty$, F_0 / c, all load is on the spring. This is shown graphically below in figures 1.5.12 and 1.5.13 where the effects of sudden load removal at $t = t_1$ is also shown.

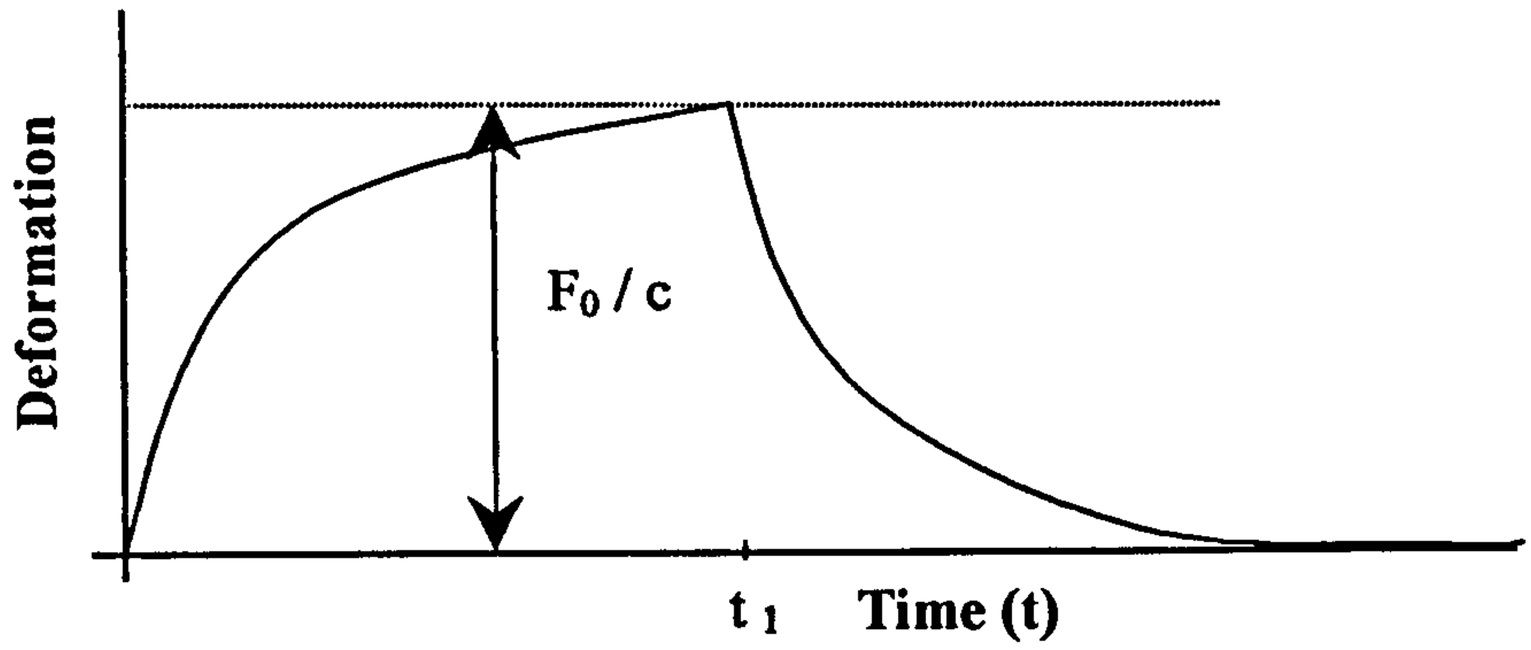

Figure 1.5.12 The deformation-time relationship for the Kelvin element at time t = 0, when the load of F_0 is applied and at $t = t_1$ when the load is removed.

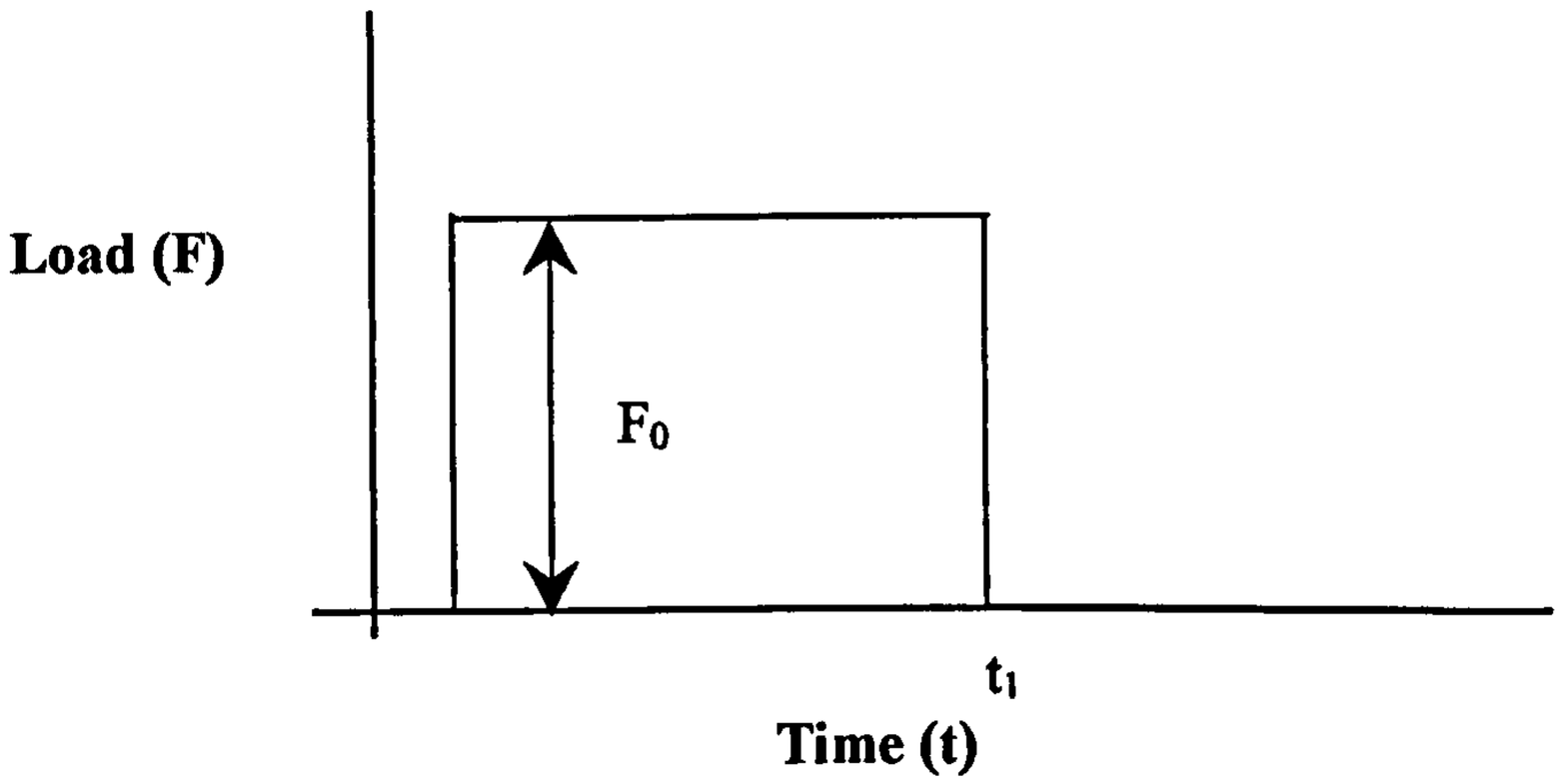

Figure 1.5.13 The load-time relationship for the Kelvin element at time t = 0, when the load of F_0 is applied and at $t = t_1$ when the load is removed.

In biological contexts, the model for an ideally viscoelastic material giving both an instant elastic response to loading and lacking plastic properties has been proposed to describe the skin. It is a combination of a Kelvin element in series with an elastic element (Hooke element) and is described diagrammatically below (Figure 1.5.14):

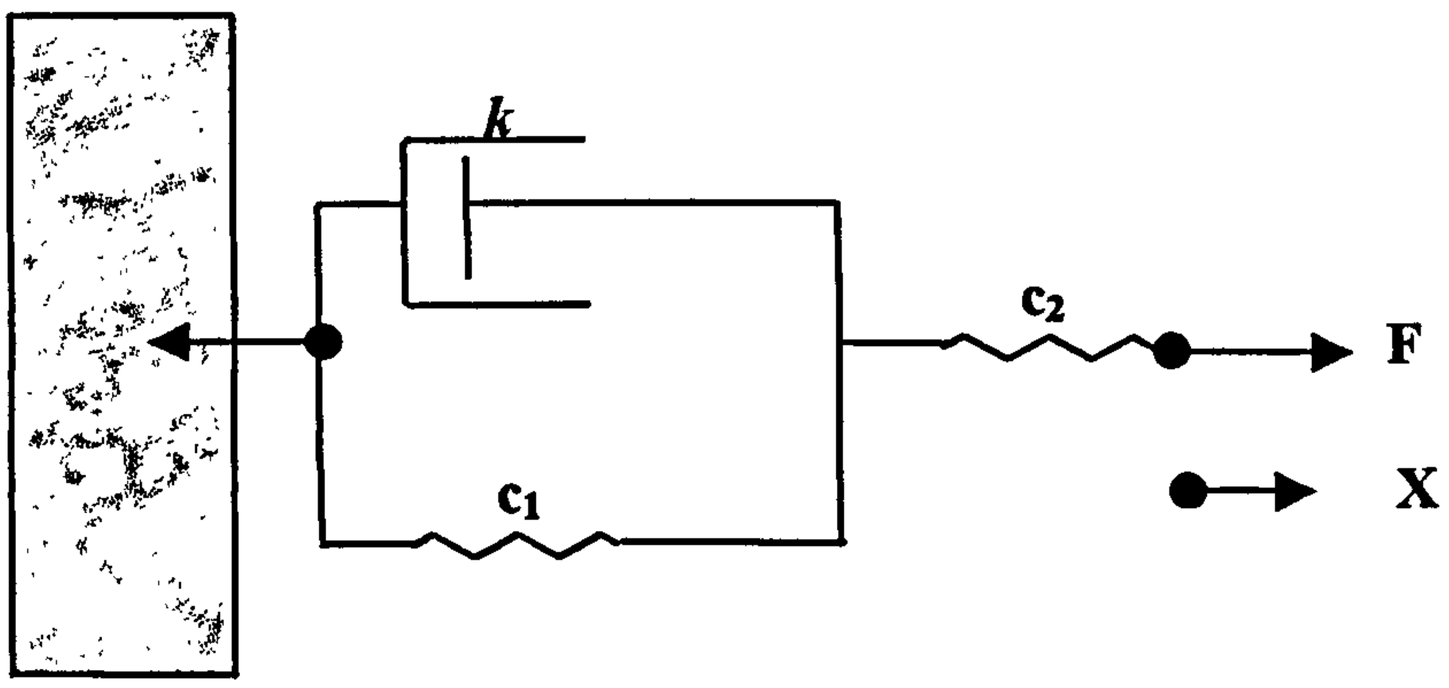

Figure 1.5.14 A model for an ideally viscoelastic material with a Kelvin and a Hooke element in series.

The above model is a simple biological model which does not take into account the plasticity exhibited by most biological materials and obeys equation 1 which is an integration of the expressions for the Kelvin-Hooke array giving the expression for deformation X as a function of time,

$$X_t = a + b\,(1 - e^{-t/\tau}) \quad \text{.....equation 1}$$

where,

X_t = distance moved at time t,

$a = F / C_2$ i.e. Force divided by spring constant of the series Hooke element

$b = F / C_1$ i.e. Force divided by spring constant of the series Kelvin element

τ (tau) = is the ratio of dashpot constant to spring constant in the Kelvin element, $\tau = k / C_1$.

After releasing the plunger with the 30g weight, the distance travelled at t=0 (X_0) is:

$$X_0 = a = F / C_2 \quad \text{.....equation 2}$$

X_0 can be read from the initial drop (i.e. phase I, the initial rapid indentation phase) and F is known, therefore, C_2, the spring constant of the series Hooke element, can be calculated.

At t=infinity, the distance X_I is equal to a + b.

$$X_I = a + b = X_0 + F / C_1 \quad \text{.....equation 3}$$

Substituting equations 2 and 3 into 1 gives the relation between distance from final equilibrium ($X_I - X_t$) and time,

$$X_I - X_t = (X_I - X_0)\, e^{-t/\tau} \quad \text{.....equation 4}$$

Equation 1 can be illustrated graphically as in figure 1.5.15 below with four theoretical curves shown with various spring and dashpot values (Bates PhD thesis 1993).

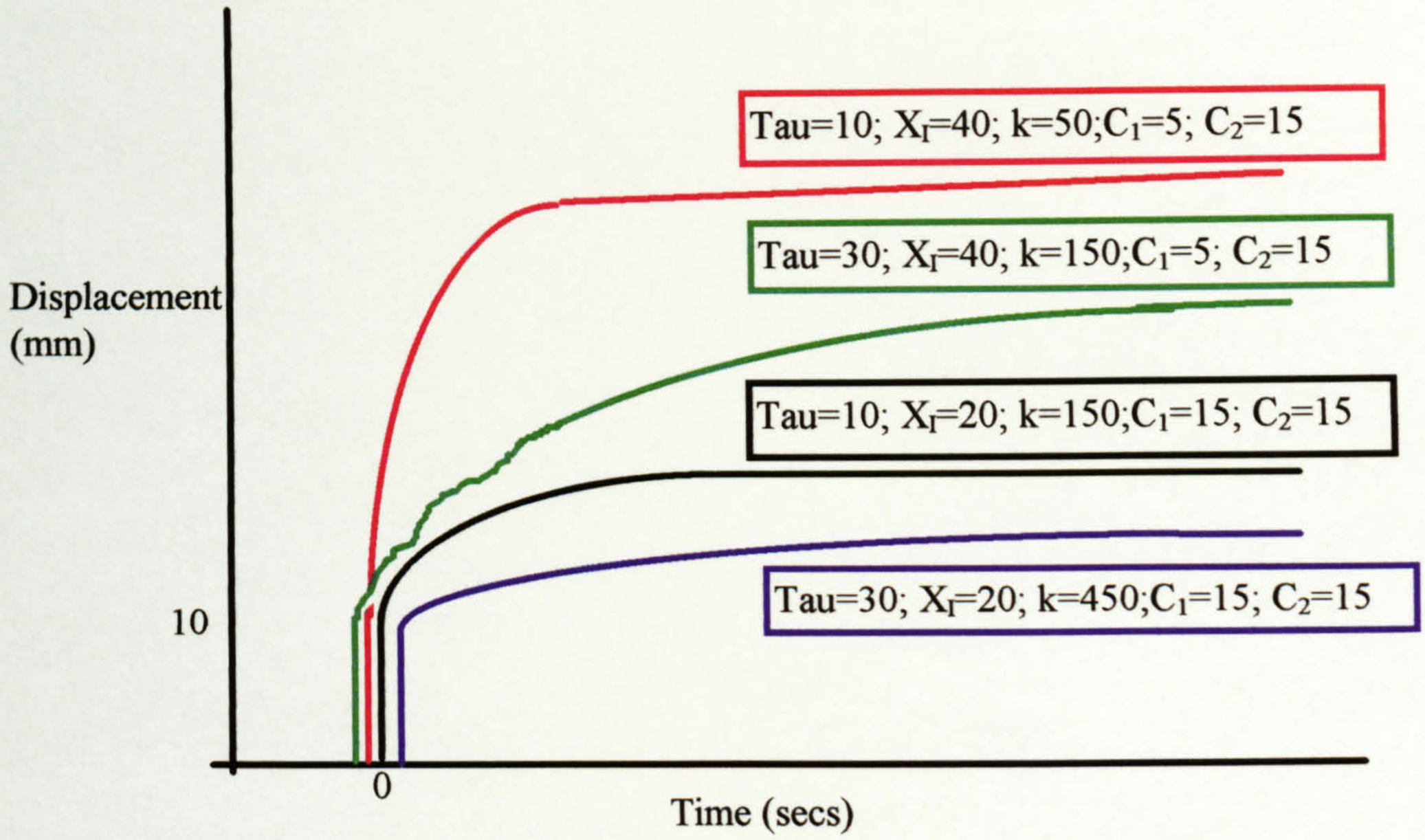

Figure 1.5.15 Theoretical tonometer curves as described by equation 1 with the values of X_0, X_I, tau, C_1, C_2 and k given.

Once t >0 seconds, the initial elastic deformation phase or phase I (initial rapid indentation phase) which is 10mm in all cases shown, has no effect on the shape of the curve. An increase in the final value that the plunger reaches (X_I) is a result of a decrease in the Kelvin elastic component, C_1. An increase in the rate at which the final value is approached results from a decrease in the rate constant.

The dashpot constant (k) is equal to the product of τ and C_1 and is proportional to the resistance to flow. An increase in the dashpot constant will therefore cause an increase in the time the plunger takes to level off.

If the final distance reached also increases, however, (decreasing C_1) then the dashpot constant may be the same (see black curve and green curve above). The dashpot constant will be greatest when both τ and C_2 are maximum, i.e. very slow pitting and to a very small depth as in the blue curve above. The dashpot constant will be lowest in very oedematous legs (red curve).

If X_I can be determined, then C_1, the spring constant of the series Kelvin element, can be calculated and thence, k the dashpot constant, can also be calculated.

From equation 4, a linear expression is obtained by taking the natural logarithm:

$$\mathbf{In\ (X_I - X_t) = In\ (X_I - X_0) - t/\tau}\text{.....equation 5}$$

By plotting In $(X_I - X_t)$ against time, a straight line should result, if the model is valid, with an intercept of In $(X_I - X_0)$, and a gradient of $-1/\tau$ called the rate constant. Because $\tau = k/C_1$, the dashpot constant, k, can be calculated once C_1 is known. X_I cannot be measured directly but can be estimated by an iterative procedure. The final recorded X_t was taken as an initial estimate of X_I. If this value was too small or too large, the semi-log plot was non-linear and correlation coefficient was low. X_I was then iteratively increased or decreased until an optimum correlation coefficient, closest to 1, was found and this was taken as the best estimate of X_I and used in the estimation of τ, C_1 and k. A simple computer curve fitting programme was designed to iteratively find the best estimate of X_I (PDCS,1996).

Interpretation of the Kelvin-Hooke model

The biological components equivalent to the two elastic and one viscous components in the above model are not specified but by analogy with other biomechanical work investigating fluid flow through cartilage (Mow et al, 1984; Armstrong et al, 1984), the initial fast indentation phase was analogous to the rapid elastic deformation of a compressible matrix and the subsequent slower indentation phase was analogous to the viscous creep of fluid through that matrix until it approaches elastic equilibrium.

Two elastic spring constants (C_2 and C_1) and the viscous dashpot constant (k) are provided by the Kelvin-Hooke model. The initial rapid deformation of a visco-elastic material, as explained by the above model, depends on its elastic component or Hooke element (spring 2), C_2, which is in series with a parallel dashpot-spring system (Kelvin element). The Kelvin element comprises a dashpot, k, and spring 1 (C_1) in parallel and this gives rise to the exponentially decaying creep until an elastic equilibrium is reached. C_2 represents the initial ability of the interstitial matrix to undergo elastic deformation without flow. The size of the subsequent deformation $X_I - X_0$ for a given load reflects the strength of the in-parallel component, C_1. The rate at which the final value is reached depends on C_1 and the resistance of the dashpot constant, k. The assumption is that k would depend on the mobility of interstitial fluid and on the interstitial hydraulic resistance which is widely accepted to be reduced in oedema. From the Kelvin-Hooke analogy, the following features would be expected:

(i) C_2 (or X_0) (g/mm) should depend only on the initial elastic compressibility and should not depend on the viscosity of the fluid in the matrix through which it is being forced.

(ii) The total depth of pitting (X_I)(mm) should depend on C_1 (g/mm) and not on the viscosity of the fluid in the matrix.

(iii) The rate constant, -1/Tau (seconds^{-1}), depends on the dashpot constant, k (g/(mm/sec), and hence on the resistance to flow as well as C_1, so k should increase with viscosity.

1.6 Methods of classification and grading of lower limb chronic venous disease.

1.6.1 Previous classification methods

Previous methods of classification of lower limb venous disease include that of Widmer (Widmer et al, 1978) which categorised limbs based on their clinical appearance. More recently, he recognised that chronic venous insufficiency must include both superficial and deep lesions regardless of their cause or their clinical presentation and suggested a five-stage chronic venous insufficiency classification (Griton and Widmer; 1992).

Hach in Germany (Hach et al, 1980) classified reflux in the long saphenous veins into four stages ranging from reflux in the proximal thigh as stage 1 to ankle reflux as stage 4. He also correlated increased superficial vein diameter, elongation and tortuosity with the progressive stages of reflux Again though this classification was popular in some countries it never achieved world-wide acceptance.

In Russia in 1985, a more detailed method based on haemodynamic and phlebographic data was described by Sytchev but this was not widely publicised outside Russia and hence never gained universal acceptance.

In the late eighties, methods of classification included the addition of an anatomic category, a clinical severity scale and recommendations for objective measurement of venous haemodynamics to document the presence of chronic venous insufficiency by the joint council of the Society for Vascular Surgery/North American Chapter, International Society for Cardiovascular Surgery (SVS/ISCVS) in their first edition of the reporting standards in venous disease

In the early nineties, another classification method from Argentina (Enrici et al, 1992) was based on the clinical presentation of the patient using anatomical regions, clinical severity, physical examination and functional assessment. This classification method was similar to the Ad Hoc Committee of the SVS/ISCVS method of classification.

More recently, Cornu-Thenard of France, described a classification system which was exclusively for varicose veins and made no attempt to classify more severe venous disorders (Cornu-Thenard et al, 1991).

Attempts to use the SVS/NA-ISCVS clinical classification described above have been disappointing as some investigators found it necessary to group stages 1 and 2, the earlier stages of the clinical manifestation of venous disease, together in order to differentiate them from the more advanced stages 2 and 3.

1.6.2 CEAP method of classification and scoring of lower limb chronic venous disease.

In 1994, an international consensus report under the auspices of the American Venous Forum presented the latest method of classification and grading of lower limb chronic venous disease which was as a result of significant progress made in the non-invasive field of investigations of venous pathology with the use of electronic and computer-aided technology. This system requirement were similar to those for diagnosing peripheral arterial disease which had long been in use.

The CEAP method classifies lower limb chronic venous disease according to the clinical signs and symptoms (C), the (a)etiology (E), the anatomic distribution of the pathology (A) and the pathophysiologic dysfunction (P) resulting in a multi-axial system (Beebe et al, 1995). The clinical classification differentiates telangiectases from truncal varicose veins, and healed ulcers from open ulcers, in a seven stage description. In addition to the classification of disease, the CEAP method also allows you to score the disease.

The CEAP method of classification is summarised in Table 1.6.1:

C for **C**linical signs (grade $_{0\text{-}6}$), supplemented by ($_A$) for asymptomatic and ($_S$) for symptomatic presentation.

E for **E**tiologic classification (**C**ongenital (E_C), **P**rimary (E_P), **S**econdary (E_S)).

A for **A**natomic distribution (**S**uperficial (A_S), **D**eep (A_D), or **P**erforator (A_P), alone or in combination).

P for **P**athophysiologic dysfunction (**R**eflux (P_R) or **O**bstruction (P_O), alone or in combination).

Table 1.6.1 CEAP classification of lower limb chronic venous disease.

Clinical classification (C_{0-6})

C is for clinical signs (grade $_{0\text{-}6}$) graded in increasing order of severity according to the objective clinical signs of disease listed in Table 1.6.2 below. Limbs with more severe signs of chronic venous disease will be placed in the higher categories and may have some or all of the findings defining a less severe clinical category. Each limb will be further characterised as symptomatic and supplemented by ($_S$) or supplemented by ($_A$) for asymptomatic.

Therapy may alter the clinical category of a limb therefore it was recommended that limbs should be reclassified after any form of medical or surgical intervention at least 6 months after.

Class 0:	No visible or palpable signs of venous disease.
Class 1:	Telangiectases or reticular veins.
Class 2:	Varicose veins.
Class 3:	Oedema.
Class 4:	Skin changes ascribed to venous disease (e.g. pigmentation, lipodermatosclerosis, venous eczema).
Class 5:	Skin changes as in class 4 with healed ulceration.
Class 6:	Skin changes as in class 4 with active ulceration.

Table 1.6.2 Clinical classification of lower limb chronic venous disease.

Etiologic classification (E_C, E_P, or E_S)

E is for etiologic classification, that is, whether the venous dysfunction may be congenital, primary or secondary (table 1.6.3).

Congenital (E_C)	
Primary (E_P)	- with undetermined cause
Secondary (E_S)	- with known cause
	Post-thrombotic
	Post-traumatic
	Other

Table 1.6.3 Etiologic classification of lower limb chronic venous disease.

Anatomic classification ($A_{S, D, P}$)

A is for anatomic distribution, that is, whether the vein(s) affected by venous disease is in the superficial, deep or perforator systems. One, two or three systems may be involved in any combination. Table 1.6.4. below lists the anatomic segments in the superficial, deep and perforator systems that may be involved.

Segment #	*Superficial Veins* (A_{S1-5}) :
1	Telangiectases / reticular veins
	Long Saphenous Vein
2	Above Knee
3	Below Knee
4	Short Saphenous Vein
5	Non-Saphenous Vein
	Deep Veins (A_{D6-16}):
6	Inferior Vena Cava
	Iliac
7	Common
8	Internal
9	External
10	Pelvic - Gonadal, broad ligament, other
	Femoral
11	Common
12	Deep
13	Superficial
14	Popliteal
15	Crural - anterior tibial, posterior tibial, peroneal (all paired)
16	Muscular - Gastrocnemial, soleal, other
	Perforating veins ($A_{P17,18}$):
17	Thigh
18	Calf

Table 1.6.4 Anatomic classification of lower limb chronic venous disease.

Pathophysiologic classification ($P_{R,O}$)

P is for pathophysiologic dysfunction which may result from reflux (P_R), obstruction (P_O) or both ($P_{R,O}$) as shown in table 1.6.5 below.

It is now recommended that in the investigation of patients with chronic venous disease, sufficient objective measurements of venous haemodynamics and anatomy by either invasive or non-invasive means must be carried out to adequately document the individual pathophysiologic changes, reflux, and/or obstruction accompanying chronic venous disease. Phlebographic or vascular laboratory investigations can objectively assess the presence of venous outflow obstruction (P_O) as well as the presence of venous reflux (P_R) in the superficial, deep and perforating systems.

The anatomic segments that are involved with either reflux or obstruction are reported using the venous segments outlined in table 4 above, as the severity of venous dysfunction is influenced by the anatomic location and extent of the reflux and/or obstruction present (Gooley et al, 1988; Hanrahan et al, 1991).

Reflux (P_R)
Obstruction (P_O)
Reflux and Obstruction ($P_{R,O}$)

Table 1.6.5 Pathophysiologic classification of lower limb chronic venous disease.

Scoring of venous dysfunction

Another feature of the CEAP method of classification of lower limb chronic venous disease is the scoring system of chronic venous dysfunction that is incorporated within the system. This provides a numerical base for the scientific comparison of limb condition and evaluation of results of treatment. The scoring system is based on three elements:

- the number of anatomic segments affected (anatomic score)
- grading of symptoms and signs (clinical score)
- disability (disability score).

This method of scoring and grading is thought to be more accurate as the grading of signs is objective whereas the grading of symptoms is subjective.

Clinical Score

The clinical score is the sum of the values assigned to the signs and symptoms listed in table 1.6.6 below:

Signs and Symptoms	*Clinical Score*
Pain	(0 = none; 1 = moderate, not requiring analgesics; 2 = severe, requiring analgesics)
Oedema	(0 = none; 1 = mild/moderate; 2 = severe)
Venous Claudication	(0 = none; 1 = mild/moderate; 2 = severe)
Pigmentation	(0 = none; 1 = localised; 2 = extensive)
Lipodermatosclerosis	(0 = none; 1 = localised; 2 = extensive)
Ulcer - Size (largest ulcer)	(0 = none; 1 = <2cm dia; 2 = >2cm dia)
Ulcer - Duration	(0 = none; 1 = <3months; 2 = >3months)
Ulcer - Recurrence	(0 = none; 1 = once; 2 = more than once)
Ulcer - Number	(0 = none; 1 = single; 2 = multiple)

Table 1.6.6 Clinical scoring of the signs and symptoms of lower limb venous disease.

Anatomical score

The anatomical score is the sum of the anatomical segments affected by either reflux, obstruction or both, each scored as one point, as listed in Table 1.6.4.

Disability score

The disability score is the sum of the values assigned to the signs and symptoms listed below in Table 1.6.7.

Score	*Level of disability*
0	- Asymptomatic
1	- Symptomatic, can function without support device
2	- Can work 8-hour day ONLY with support device
3	- Unable to work even with support device.

Table 1.6.7 Disability Score.

1.6.3 Previous applications of the CEAP method

This method has been published in 19 different journals and books in 5 languages around the world.

This classification has been used in the subfascial endoscopic perforator study (SEPS) in which they classified data of patients from 17 medical centres (Gloviczki et al, 1997). They found the disability scoring method very valuable, as calculated preoperatively and postoperatively, in giving a clear indication of the results of the intervention.

In a crossover study investigating the controversy of non-operative versus operative therapy for the treatment of the complications of venous hypertension it was found that the clinical severity scoring was very useful in comparing the results pre- and post-operatively (DePalma et al, 1996).

1.7 Normal anatomy of the lower limb venous system

Venous blood flow is influenced by physical factors such as pressure and resistance and is also dependent upon gravity, cardiac cycle, respiratory cycle, blood volume and the presence of the calf muscle pump which is often referred to as a 'peripheral heart'.

1.7.1 Structure of the vein

The veins consists of three layers: the intimal, medial and adventitial layers. The walls of the veins are on average much thinner than those of arteries. The intima is composed of a monolayer of endothelial cells with an underlying basement membrane and elastic lamina. The media consist of three layers of smooth muscle with interspersed elastic and connective tissue. The adventitia is histologically, the thickest layer of the vein wall. It is composed of interlacing fibres of collagen and contains the venous vasa vasora and the peri-venous adrenergic nerve fibres.

The capacitance function of the venous system is primarily due to the thin, collapsible vein walls. The optimal function of the calf muscle pump is dependent on high venous capacitance. Duplex ultrasound B-mode scanning can clearly demonstrate the resulting elliptical configuration of a collapsed vein when subjected to external pressure. This pattern of collapse and re-expansion in response to external compression contributes to the high capacitance of the lower extremity venous system. Most of the veins in the lower extremity lie within the muscular compartments of the leg and are subject to external compression during calf muscle contraction. When veins fill and expand, they change from an elliptical to a circular configuration which results in an increase in venous volume without an increase in circumference of the vein. As the vein wall is not stretched, there is no resulting increase in pressure or energy expended to fill the vein. Once the vein reaches a circular shape further increases in venous volume require increased intra-luminal pressure to increase the venous circumference and venous volume. Because of the thinness of the vein walls, minimal pressure increases are needed to overcome the inherent stiffness of the adventitial layer. The venous volume is increased by over 250% as a result of an increase in the venous transmural pressure from 0 to 15 mmHg (Moreno et al, 1970). Using Duplex ultrasound B-mode scanning to observe the large axial veins that are not subject to muscular compression, it was found that they collapsed in a circular rather than elliptical configuration is response to a decrease in venous volume (Moneta et al, 1988). These veins are supported on all sides by connective tissue and/or fat and are subject to equal external pressure vectors at all

points on the vein wall and expand or collapse in response to changes in venous volume through changes in circular diameter rather than change in the shape of the vein resulting from external compression.

1.7.2 The venous valves

Valves are present in lower extremity veins and are bicuspid with a fine connective tissue skeleton and are extremely strong even though they are just a thin layer of collagen fibres covered with endothelium (Ackroyd et al, 1985). The valve cusps are stronger and more elastic than the vein wall. The valve sinus is always wider than the vein above and below the cusps (Cotton et al, 1961). The calf muscle pump (described in the next section) in combination with functioning venous valves provides the force necessary to overcome venous compliance and hydrostatic pressure and promote venous return in the upright position. There are more valves in the distal leg veins and they decrease in numbers proximally. The inferior vena cava and common iliac veins have no valves and 75% of external iliac veins have no valves, but only 25% of common femoral veins are valveless (Powell et al, 1951). Valves in the internal iliac systems are less frequent, occurring in about 7% of the population (Basmajian et al, 1952). It has been suggested that the lack of valves in the iliac and common femoral veins is the starting point for the development of a progressive descending valvular incompetence that causes varicose veins (Ludbrook et al, 1962). The valves in the axial veins ensure that blood flows from caudal to cephalad and from superficial to deep in the communicating veins of the calf with the exception of foot communicating veins where venous flow is from the deep veins in the muscles of the sole of the foot to the superficial veins on the dorsum (Gardner et al, 1983), (Pegum et al, 1967). The valves close in response to cephalad to caudal blood flow and a reverse flow velocity of at least 30 cm/s appears to be required for venous valve closure (van Bemmelen et al, 1990).

1.7.3 Superficial venous system

The lower extremity veins can be divided into three types: superficial, deep and perforating veins (see figure 1.7.1). The superficial venous system lies above the investing fascial layer of the leg and thigh and consists of the long and short saphenous veins and their tributaries. These veins are not compressed by the muscle contraction. The long saphenous vein (LSV) and short saphenous vein (SSV) referred to throughout in this thesis are also known as the greater saphenous vein (GSV) and the lesser

saphenous vein (LSV) respectively in American textbooks (please note to avoid confusion). The venules which are the smallest veins are present in all tissues. These join to form medium sized veins, which empty into the main trunks of the long and short saphenous veins.

The long saphenous vein

The long saphenous vein (LSV) is the longest vein in the body and it begins in the dorsum of the foot arising from the medial aspect of the dorsal pedal venous arch and ascends cephalad anterior to the medial malleolus. From the ankle, it follows a course immediately superficial to the anteromedial aspect of the tibia and at the level of the knee it lies about 8-10 cm dorsal to the medial edge of the patella. The saphenous nerve accompanies the vein anteriorly from the dorsum of the foot to the subsartorial canal in mid-thigh. The LSV remains superficial in the mid-thigh and moves deep to Scarpa's fascia in the upper thigh to enter the fossa ovalis, about 4 cm inferior and lateral to the pubic tubercle. Normally, the diameter of this vein is 2mm to 3mm in the calf and 6mm in the thigh, as seen on the venogram. The LSV contains between 10-13 valves and is duplicated in the thigh in an estimated 8% of patients with the duplicated segments reuniting at variable points distally in the thigh (Thompson et al, 1979).

Tributaries of the long saphenous vein

Important tributaries of the LSV include the posterior arch vein which arises from calcaneal branches posterior to the medial malleolus and courses cephalad in the posterior medial calf and unites with the LSV in the proximal calf.

In the proximal third of the thigh, the LSV has major anterolateral and posterolateral tributaries joining it. These tributaries may be significantly varicose without clinically evident changes in the LSV. At the level of the fossa ovalis, the LSV has constant tributaries present which include the superficial circumflex iliac, the superficial epigastric, and the superficial external pudendal veins. These tributaries have variable courses and anatomical orientation to the LSV and are best identified at the level of the saphenofemoral junction. These tributaries may become varicose and can contribute to venous reflux.

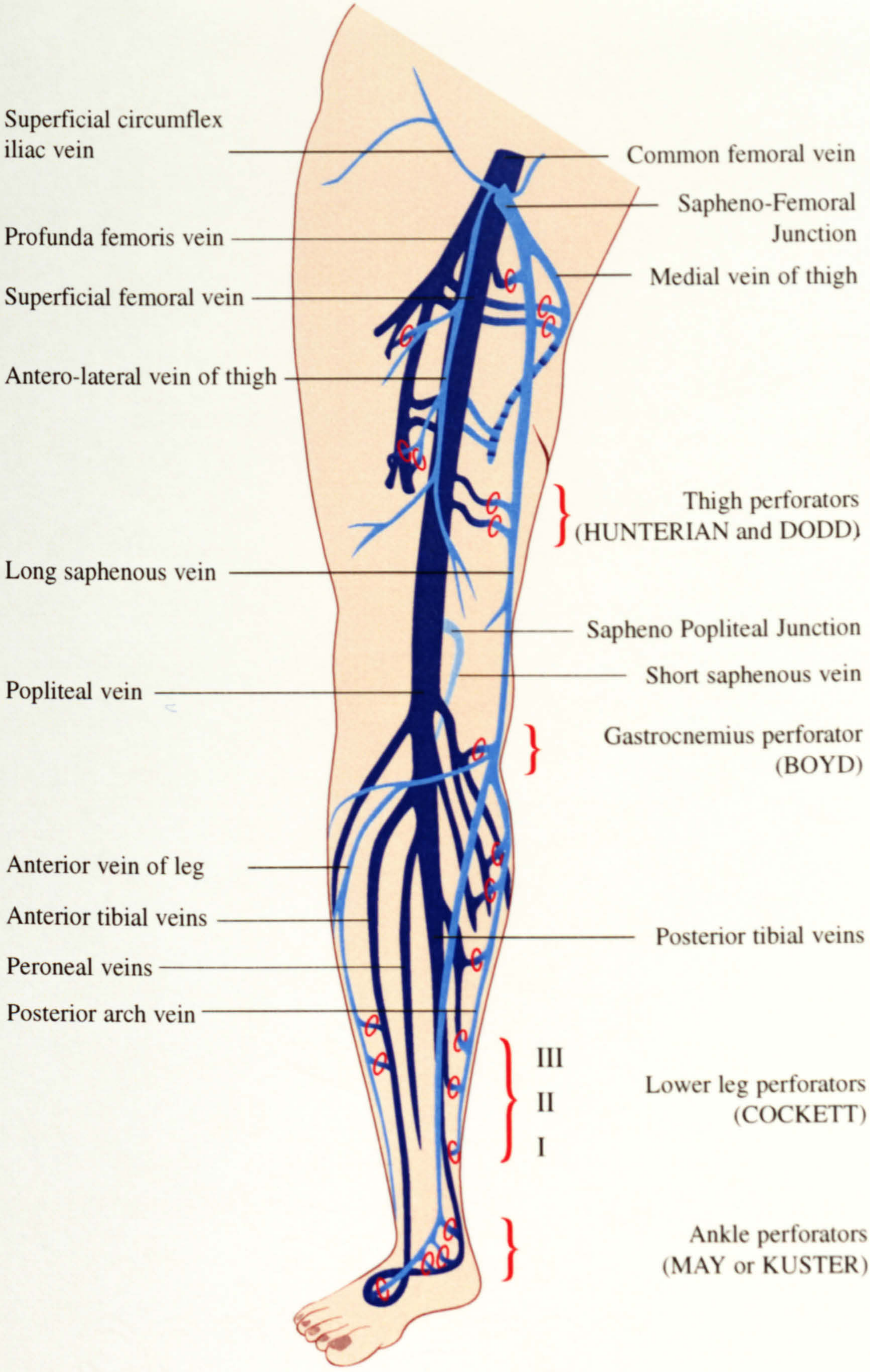

Figure 1.7.1 Normal venous anatomy of the lower limb showing the superficial, deep and perforator systems.

Communicating or perforating veins associated with the long saphenous vein

There are multiple communicating or perforating veins present between the posterior arch vein and the posterior tibial vein and are known as Cockett's perforators.

Boyd's perforator connects the LSV to the deep veins approximately 10cm below the knee. The LSV lies immediately superficial to the tibia distally and so it does not directly communicate with the deep venous system in the distal leg but is indirectly connected to the deep system via perforating veins linking the posterior arch and posterior tibial veins.

Connections exist between the LSV and the deep venous system in the thigh. The Dodd and Hunterian perforating veins connects the LSV and the superficial femoral vein approximately 15cm and 30cm proximal to the knee. Incompetence of this vein may be the aetiology of medial thigh varicosities in the presence of a competent saphenofemoral junction and a competent proximal LSV. There are also smaller communicating veins that may connect the LSV to the deep femoral vein, also known as the profunda femoris vein, in the middle third of the thigh. Many other communicating veins may exist in the LSV system and some may pass through muscle during their course.

Short saphenous vein

The short saphenous vein (SSV) originates laterally from the dorsal venous arch, travelling cephalad subcutaneously in the mid-posterior calf between the medial and lateral heads of the gastrocnemius muscle where it penetrates the deep fascia, usually in the upper third of the calf, to enter the popliteal fossa. Venographically, the diameter of the short saphenous vein normally measures up to 7mm and contains between 7-13 valves and are more closely spaced than those in the LSV. The sural nerve lies immediately lateral to the SSV. The termination of the SSV is highly variable. It can enter the popliteal vein distally to the knee joint and it may do so proximally and on occasion continues subcutaneously and joins the LSV in the thigh. The intersaphenous vein links the SSV and LSV and it courses cephalad and medial to join the LSV directly, or indirectly, by way of the posterior arch vein.

Tributaries of the short saphenous vein

The main tributary of the SSV is the lateral arch vein which courses cephalad along the lateral calf and joins the SSV just distal to the popliteal fossa. Perforating veins typically

connect the lateral arch vein to the peroneal vein of the deep venous system. This is analogous to the posterior arch vein and its relationship to the LSV.

1.7.4 Deep venous system

The deep veins lie beneath the fascial covering and are subject to compressive forces when the muscles of the limb are used. Venous return from the lower extremities is primarily through the deep veins.

Venae comitantes

There are three sets of paired venae comitantes paralleling the course of the three named arteries beginning in the distal calf. They are the peroneal, posterior tibial and anterior tibial veins. The paired veins are connected by venous bridges along the course of each artery with the paired veins joining at the mid-calf level. From this level, the peroneal, anterior and posterior tibial veins continue cephalad with their respective arteries. The anterior tibial vein drains the anterior compartment, the posterior tibial vein drains the posterior compartment, and the peroneal veins drains the lateral compartment of the leg. They join to form the popliteal vein in the inferior aspect of the popliteal fossa.

Popliteal vein

The popliteal vein lies 1 to 2cm from the posterior surface of the distal femur. The close relationship of the popliteal vein to the femoral condyles has been implicated as a cause of the pseudo-obstruction of the popliteal vein seen on venography. The normal diameter of the popliteal vein is 9 to 15mm when measured on anteroposterior radiographs at a point 8cm above the knee joint. In the popliteal vein, up to 2 valves may be present. Distally, the popliteal vein proceeds medial to the popliteal artery and proximally it courses lateral to the artery at the adductor opening and becomes the superficial femoral vein. The popliteal vein may be single, double with two femoral veins or triple.

Superficial femoral vein

The superficial femoral vein lies initially lateral in the distal thigh and then medial to the superficial femoral artery in the proximal thigh. It measures 0.9 to 1.0cm in diameter and usually contains two to five valves. Four variations of the superficial femoral vein consist of a normal single vein, duplication of the lower segment, sequentially uniting

to form one single vein in the mid thigh, multiple veins and duplication of the entire superficial femoral vein.

Deep femoral vein

The deep femoral vein, also known as the profunda femoris vein, drains the thigh muscles and it joins the superficial femoral vein laterally in the proximal femoral triangle to form the common femoral vein which lies medial to the common femoral artery. The long saphenous vein joins the common femoral vein about 3 cm distal to the inguinal ligament to form the sapheno-femoral junction. At the inguinal ligament, the common femoral vein becomes the external iliac vein and it receives tributaries from the deep epigastric and deep circumflex iliac veins. In the deep venous system, there are many hundreds of valves present with the greater number of valves present in the more distal vessels.

1.7.5 Intramuscular venous channels

The soleus and gastrocnemius are the two muscle plexus veins that can be seen on venography. The intramuscular venous channels are very important to the function of the calf muscle pump (see section 1.8.5). The numbers of venous sinuses present within the substance of the soleus muscle vary between 1-18. The soleal sinuses are valveless but are linked by valved small venous channels that prevent reflux. The soleal sinuses empty into the posterior tibial vein in the proximal calf. The soleus plexus veins are seen as saccular venous structures in the posterior calf region when viewed on the lateral venographic projection. There is also within the substance of the gastrocnemius muscles an interlacing valved venous network which coalesce to form a pair of venous channels, the gastrocnemius veins, that drains the blood from the gastrocnemius muscle and empty into the popliteal vein. These are seen as saccular venous structures in the medial calf region when viewed on the anteroposterior venographic projection.

1.8 Normal venous physiology of the lower limbs.

There are many factors that influence the return of blood from the lower extremities to the heart and the relative importance of each factor varies with body position and activity.

1.8.1 In the supine position

When the subject is in the supine position, the respiratory cycle plays an important part in the venous return from the lower extremities. The foot vein pressure in the supine position is approximately 15 mmHg. The right atrial pressure is normally between 0 and 2 mmHg so the venous return to the heart in the supine position is generated by a pressure gradient of 13-15 mmHg (Browse and Burnand, In: Diseases of the veins, 1988 p 53). During inspiration, the volume of the veins of the thorax increases and the pressure decreases in response to reduced intrathoracic pressure (Moreno et al, 1967; Moreno et al, 1969). Expiration leads to the opposite effect, with decreased venous volume and increased pressure. The venous response to respiration is reversed in the abdomen, where the pressure increases during inspiration because of the descent of the diaphragm, and decreases during expiration as the diaphragm ascends. Increased abdominal pressure during inspiration decreases pressure gradients between peripheral veins in the lower extremities and the abdomen, thus reducing flow in the peripheral vessels (Duomarco et al, 1954). During expiration, when intra-abdominal pressure is reduced, the pressure gradient from the lower limbs to the abdomen is increased and the flow in the peripheral veins rises correspondingly. The respiratory effects are usually associated with clear phasic changes in venous flow in the extremities. These can be detected by various instruments, including many forms of plethysmography and Doppler flow detectors. The respiratory changes in venous velocity may be exaggerated by respiratory manoeuvres, such as the Valsalva manoeuvre, which increases intra-thoracic and abdominal pressures and decreases, abolishes, or even reverses flow in some peripheral veins.

1.8.2 From the supine to the upright position

When the subject moves from the supine position to the upright position, the influence of the respiratory cycle changes and the effect on the venous blood flow patterns has been demonstrated in the common femoral vein using a duplex ultrasound scanner with simultaneously recordings of the cardiac and respiratory cycles using an

electrocardiogram (ECG) monitor and a respiratory monitor (Moneta et al, 1988). The subject was placed in the supine position and non-weightbearing on a tilt table and gradually moved until fully upright. At minus 10 degrees head down, the weight of the abdominal viscera presses on the diaphragm and this is sufficient to counteract the descent of the diaphragm during inspiration. In this position, the outward movement of the chest wall generates the negative intra-thoracic pressure that is required for inspiration with little change in the intraabdominal pressure. The venous blood flow patterns were shown to be primarily dependent upon the cardiac cycle but virtually independent of the respiratory cycle. Blood leaving the legs essentially flows downhill into the passively dilating right atrium until it is stopped by the onset of the cardiac contraction. As the subject becomes more upright, the influence of the cardiac cycle on the venous return rapidly diminishes as the respiratory cycle becomes more important. It has been shown in the study above that with the subject in the non-weightbearing and 30 degree head-up position on a tilt table, the respiratory monitor indicated presence of flow in the common femoral vein during expiration and ceases with normal inspiratory effort.

1.8.3 In the fully upright position

In the upright position, the column of blood between the level of the manubrium sterni, the point used as the zero reference for the pressure measurement, and the foot exerts a gravitational force known as the hydrostatic pressure. The pressure in the foot vein then becomes 15 mmHg plus the hydrostatic pressure. In this position, the expiratory promotion of venous flow is not enough to overcome the gravitational hydrostatic forces and promote adequate venous return. The contraction of the calf muscles is required to overcome this gravitational forces (see section 1.8.5) and promote venous return. The diagram below (figure 1.8.1) shows the arterial and venous blood pressure in a 6ft man in the standing position.

1.8.4 The return of blood to the heart

Blood returning from the lower extremity must flow against the forces of gravity to return to the right atrium. The foot vein pressure in the supine position is about 100 mmHg, so in order for blood to return from the foot, it has to reach this pressure to ensure adequate return. Since the vascular system is a closed series of tubes, the return pressure on the venous side is balanced by an equal hydrostatic arterial pressure

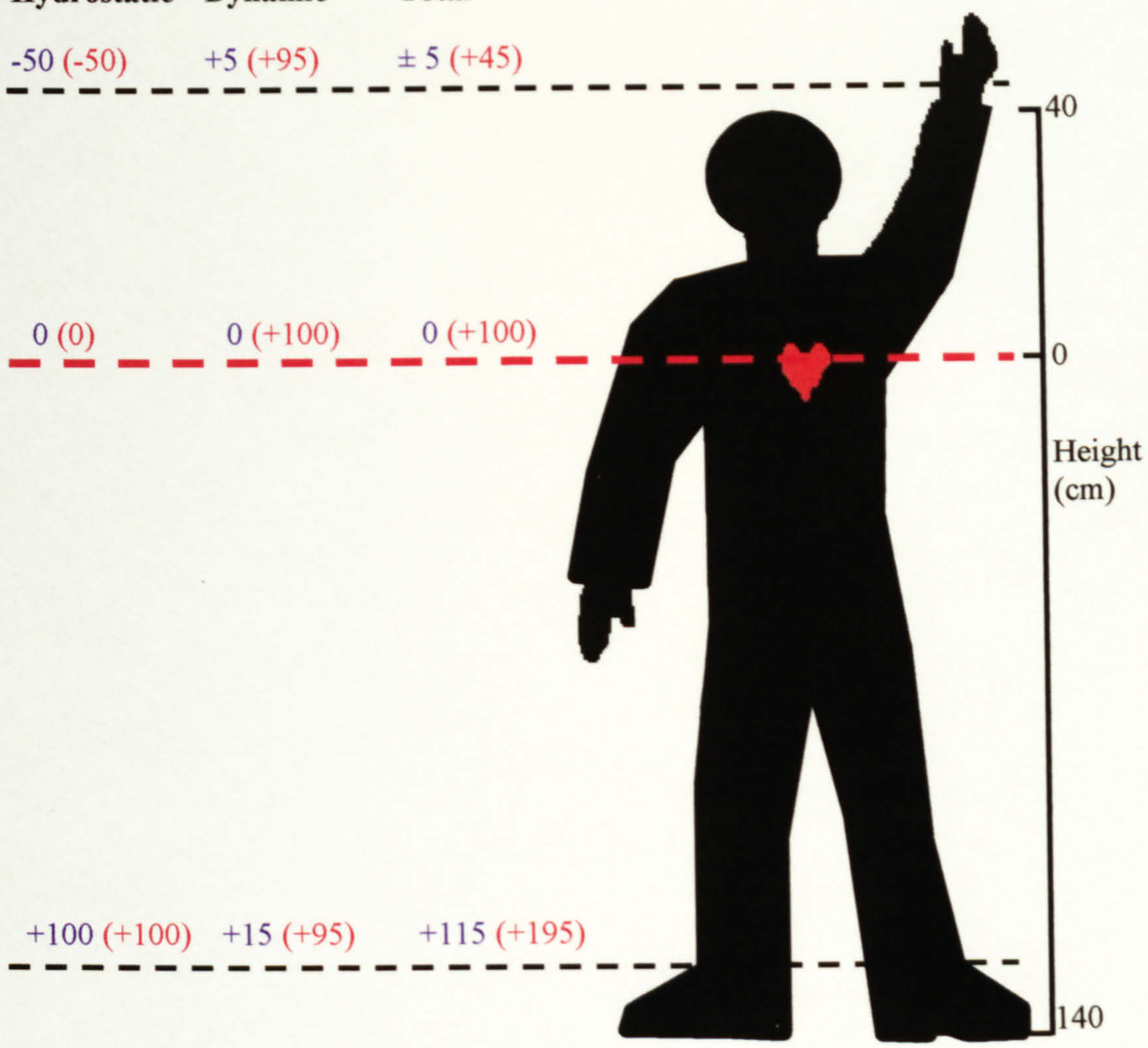

Figure 1.8.1 The arterial blood pressures (in red) and venous blood pressures (in blue) in a 6ft (180 cm) tall standing man. The pressure at any point in the circulation is the sum of the dynamic pressure, which comes from the heart, and the hydrostatic pressure, which is exerted by the column of blood between the site of measurement and the zero reference point. The heart is used as the zero point when measuring the pressures.

supplying the limb. The pumping force of the heart results in the blood leaving the capillary system having a small residual pressure of 15-20 mmHg, the 'vis a tergo'. This is sufficient to ensure venous return whether in the supine or erect position. In the supine position, the venous return takes place at the expense of a high venous pressure in the extremity. A highly specialised pumping mechanism has been evolved to actively pump venous blood back to the heart from the lower limb. It is thought that the principal participants in the pumping mechanism are the calf veins, gastrocnemius and soleus, within the calf muscles. This mechanism will be described later on in the chapter. Failure of this system results in symptoms ranging from varicose veins to severe ulceration of the lower limb.

1.8.5 The calf muscle pump

It is thought that the principal participants in the pumping mechanism are the veins of the calf within the calf muscles, gastrocnemius and soleus. These contain as much as 250ml of blood (Ludbrook et al, 1964). Additional muscle pumps are also recognised in the thigh and foot (Gardner et al, 1983) (Gardner et al, 1989 book). The latter has a much smaller capacity (about 25ml), but assists in the return of blood from the foot. It may have a 'pump-priming' effect on the calf muscle pump. The calf pump is often referred to as the peripheral heart. Venous flow is also dependent upon the contractile force of the heart, static filling pressure and gravity. During calf contraction, the pressure in and around all the structures contained within the deep fascia becomes raised resulting in all the intra-muscular veins becoming completely compressed resulting in the blood that is present in these veins being emptied by the calf muscle pump into the outflow tract which is the popliteal vein. The large veins within the gastrocnemius and soleus muscles form the main chamber of the pump but all the other deep veins participate. Distal deep venous valves prevent axial reflux and valves in the communicating veins prevent reflux from the deep venous system to the superficial venous system (Almen et al, 1962). The average volume of the calf varies between 1500 - 2000 ml and the calf blood volume is between 60 - 70 ml (Whitehead et al, 1983). With continuous exercise, the calf blood volume is reduced by 1.5 - 2.0 ml/100ml mainly as a result of the compression of the veins in the pump chamber and the average expelled volume is approximately 30 - 40 ml, that is, about 50% of all the blood within the pump. The pump will normally expel this volume in four or five contractions though one single sustained contraction can expel as much. The popliteal vein is a large bore

vein which offers virtually no resistance to outflow. As the gradient of 10 - 15 mmHg between the small veins and the heart is sufficient to ensure venous blood flow in the supine position, the increase in gradient produced by the calf muscle pump during contraction is more than enough to ensure an adequate rapid venous return to the heart during vigorous erect muscle exercise.

As the calf muscles relax, valves cephalad to the muscle pump close and prevent reflux of blood from more proximally in the axial veins. The valves in the communicating veins open and allow normal blood flow from the superficial to the deep venous system. In addition, arterial inflow from the lower extremity capillary beds re-primes the calf muscle pump for a subsequent contraction. At the moment when the calf muscles relax their contained veins are empty, at low pressure and as yet unfilled by arterial inflow. As the veins are collapsed, they are unaffected by hydrostatic pressure. On the other hand, the superficial veins are full and subjected to hydrostatic pressure plus the remnant of cardiac generated pressure, the 'vis a tergo'. The pressure gradient between the two compartment becomes 100 - 110 mmHg resulting in blood immediately flowing from the superficial to the deep compartment through the many communicating veins (Bjordal et al, 1970). This empties the superficial compartment and reduces its pressure (Pollack et al, 1949). Therefore the function of the calf muscle pump is vital in ensuring venous return from the lower limbs during exercise and the reduction of the superficial venous pressure thus removing the damaging effects of the hydrostatic pressure ever present as a result of man's upright posture.

The effectiveness of the calf muscle pump can be investigated by air plethysmography and ambulatory venous pressure measurement technique which measures deep venous pressure directly by placing a needle in a dorsal foot vein and connecting it to a standard pressure transducer and a recording device during rest and exercise.

1.9 Lower limb venous pathophysiology

The pathophysiology of chronic venous disease is influenced by changes in the cutaneous microcirculatory physiology as well as macrocirculatory changes. These changes play an important role in the venous ulcerative process with the skin and subcutaneous tissues in the distal third of the lower limb, mainly in the gaiter area, acting as the final target organs of chronic venous insufficiency. The hydrostatic and ambulatory venous pressures are highest in this area as a direct result of venous hypertension due to valvular reflux, with the high venous pressures being transmitted

from the macrocirculation to the microcirculation which may ultimately result in oedema, skin changes and venous ulceration.

1.9.1 Valvular reflux or retrograde flow

This is the return of blood flow in the opposite direction from physiological flow which is centripetal. This reflux may be natural, physiological or pathological. The duration of the reflux flow depends on the physiological or pathological state of the valvular apparatus. There have been studies (van Bemmelen et al, 1989) to show that the duration of reflux in the deep venous trunks in normal subjects was less than 0.5 seconds in 95% of subjects scanned. Other studies (Neglen et al, 1992; Neglen et al, 1993) have also shown that reflux is significant if the duration of the retrograde flow within the veins exceeds 0.5 seconds. Although it is not necessarily correlated to the development of varicose disease, this minimum duration of 0.5 seconds does seem to be universally recognised as corresponding to pathological reflux (Sarin et al, 1992; Moulton et al, 1993; Coleridge Smith et al, 1993). Kistner proposed a classification of deep vein reflux according to the level of descent of radiological contrast medium, during descending venography (Kistner et al, 1978; In: Venous problems). He defined four classes in increasing order of severity, with class 1 corresponding to reflux descending to mid-thigh, class 2 corresponding to reflux descending to the knee, class 3 corresponding to reflux descending to the mid-calf and class 4 corresponding to reflux descending to the ankle.

There are several ways of eliciting reflux flow in the lower limb venous system and the most effective ones includes manual compression of the calf or by means of a cuff.

Compression with the cuff placed on the calf and inflated to 120 mmHg and then rapidly deflated thus mobilising the blood from the superficial and deep veins is the method that is easy to reproduce systematically since automatic cuffs which can inflate and deflate rapidly are available. A rapid cuff inflator and cuff deflator device was built by the author (see section 2.7) to standardise all cuff compression studies to elicit antegrade flow in reflux quantification studies performed in this thesis and has been reported by others (van Bemmelen et al, 1989; Vasdekis et al, 1989).

The accurate quantification of venous reflux in individual veins has not yet been fully established. Recently, there have been several methods of quantifying venous reflux in individual veins using duplex scanning. These include measurements such as valve closure time (Welch et al, 1992), venous reflux index or the Doppler efficiency index

(EId) (Beckwith et al, 1993), velocity at peak reflux and quantitative volume flow measurements by calculating the cross-sectional area of the vessel and the time-averaged velocity (Vasdekis et al, 1989). Experience with these methods are still limited (Araki et al, 1993).

The haemodynamic studies, using the duplex scanner, are essentially that of reflux and will depend on several factors which can be standardised to provide useful information. However, this method can only measure one venous segment at a time.

Several methods have been developed to assess venous reflux but ambulatory venous pressure measurements, though invasive, is still considered the 'gold standard' (Raju et al, 1990). However, duplex scanning is the non-invasive method of choice used for testing individual veins of the superficial, deep and perforating systems (van Bemmelen et al, 1989; Neglen et al, 1992; Welch et al, 1992; Szendro et al, 1986; Vasdekis et al, 1989). Duplex scanning of patients in the upright position in combination with proximal deflation of a venous-occluding blood pressure cuff to augment flow in the veins is the best documented non-invasive method of quantifying reflux by measuring reflux duration in specific axial superficial or deep venous segments (Masser et al, 1995). In addition, duplex scanning can detect the presence of incompetent perforating veins (Hanrahan et al, 1991; Hanrahan et al, 1991). It can also determine the anatomy of veins in the popliteal fossa (Hobbs et al, 1986; Vasdekis et al, 1989; Engel et al, 1991). In the presence of deep venous disease, duplex scanning will determine whether the problem is due to anatomic obstruction, reflux or both, and the anatomic extent of the pathology.

Quantifying the duration of reflux on duplex scanning provides a valid method for giving indications but is dependent on the endoluminal diameter and on the manoeuvre performed. The manoeuvre can be standardised by using standardised calf compression to elicit antegrade flow with an automatic cuff inflator and deflator using the methodology described by van Bemmelen (van Bemmelen et al, 1989).

1.9.2 Oedema

Starling forces and how oedema is formed

Oedema is described as a perceptible expansion of the interstitial fluid volume. While the clinical importance of oedema is usually that of its underlying cause, the accumulation of oedema fluid and elevation of tissue pressure may directly interfere with various physiologic activities.

Most of the fluid that enters the tissue space by ultrafiltration from the arterial end of the capillary bed is absorbed back into the circulation at the venous end of that network. Ernest Starling, in 1896, described interstitial fluid formation and absorption in terms of the pressure gradients acting across the capillary endothelium, the surface area available for fluid transfer and the permeability of the capillary membrane to protein. The direct and immediate causes of oedema are alterations in the Starling forces that lead to an increase in the capillary-absorption ratio and a decrease in lymphatic drainage.

Oedema is observed in a wide variety of diseases in which one or more of the Starling forces are modified by the disease process. In most cases, there is a complex interaction among the factors that control transcapillary fluid exchange and interstitial volume. A simple example of oedema is the orthostatic oedema that occurs in the dependent parts of the body. In this case, gravity acts to impair venous return resulting in increased capillary filtration rate as a result of increased capillary volume and capillary pressure. The single affected element of the system is the capillary pressure. Any procedure that will facilitate venous return such as muscle activity or compression stockings will alleviate the condition.

In lower limb chronic venous insufficiency, oedema may occur as a result of increased capillary pressure secondary to an elevation in venous pressure due to venous obstruction or increased venous hypertension due to valvular incompetence. Other causes of clinical oedema include pregnancy, congestive heart failure, renal or hepatic insufficiency.

Interstitial glycosaminoglycan (GAG) concentration and viscosity.

Normal interstitium consists of fluid within a GAG and collagen matrix. The presence of quasi-fixed very large GAG molecules in the interstitium contributes significantly to the resistance to flow of fluid through the interstitium (Levick et al, 1987).

In oedema, pools of freely mobile interstitial fluid (oedema fluid) exist in contact with the neighbouring interstitial matrix (Guyton et al, 1971). Leaching of GAGs into the free interstitial fluid pools might increase the viscosity of the oedema fluid and may make it more difficult for fluid to be removed from the tissue spaces.

Interstitial protein concentration in lymphoedematous tissues is considered higher than other oedematous states. Therefore, lymphoedema is a high protein oedema.

In venous oedema of the lower limb, the interstitial fluid protein concentration was found to be 7.3g/l and in a lymphoedematous upper or lower limb as a result of surgery it is between 21-36g/l (Crockett et al, 1956).

Schematic representation of skin

An anatomical model of skin has been previously described (Guyton et al, 1971) to characterise the rate of flow on compression as a function of two viscosities: that of the free fluid and that of the gel-like fluid (see figure 1.9.1).

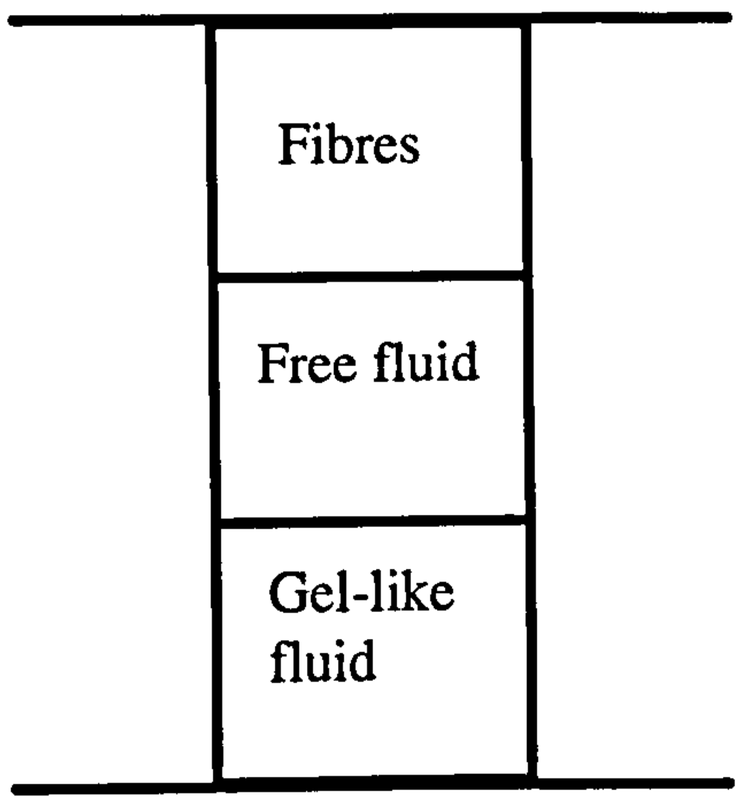

Figure 1.9.1 Schematic representation of the anatomical model of skin.

It has been previously demonstrated that the mobility of fluid flow in the subcutaneous tissue increases substantially with oedema (Guyton et al, 1971). The fluid flow through the tissue spaces is normally restricted by solid elements and by the high viscosity of the gel-like matrix embedded in a network of collagen fibres (Brace and Guyton, 1979). As oedema develops, the tissue spaces become greatly enlarged and fluid replaces the gel in the tissue spaces. The mobility of the interstitial fluid should indicate the degree of oedema.

1.9.3 Skin changes and venous ulceration

Haemosiderosis

Venular dilatation occurs after prolonged venous hypertension resulting in extrusion of red blood cells through the interendothelial pores in post-capillary venules. The red blood cells containing haemoglobin are broken down, leaving haemosiderin as a brown pigment which stains the skin mainly on the lower medial third of the lower limb. This staining may slowly spread around the leg to cover the entire 'gaiter' area. The

pigmentation may get darker and eventually look almost black (Browse and Burnand, 1988).

Lipodermatosclerosis

Lipodermatosclerosis (LDS) has been described as the progressive fibrosis of the skin and subcutaneous tissues induced by chronic venous hypertension resulting in skin pigmentation and thickening and hardening of the skin and fat (Browse et al, 1983). LDS is characterised by skin induration and hyperpigmentation of the legs that often occurs in patients with chronic venous insufficiency. One study showed that 88% of ulcers and liposclerotic skin changes were located in the gaiter area of which 76% were on the medial side (Callam et al, 1992). Although it has not been demonstrated that all patients with LDS have venous insufficiency, most authors agree that LDS appears to be highly associated with or restricted to the legs of patients with chronic venous insufficiency. LDS is thought to consist of an acute inflammatory stage followed by a chronic stage characterised by extensive fibrosis or sclerosis. The acute phase of LDS has the clinical features of pain and discomfort in the leg above the medial malleolus. The area is erythematous, scaling, tender and warm. At this stage, some induration is already present though the sharp demarcation of hardness noted in chronic LDS is usually absent (Kirsner et al, 1993).

In chronic LDS, the skin is stiff and shiny and fixed to the hard indurated contracting subcutaneous tissues with a palpable edge and the skin appears brown and shiny. The leg appears to look like an inverted 'champagne bottle' due to the oedema of the calf above and the progressive contraction of the skin and subcutaneous tissues which results in shrinkage of the gaiter area.

1.9.4 Pathophysiology of venous ulceration

The physiologic abnormalities accompanying the microcirculatory changes in chronic venous insufficiency are not well defined. There are three prominent theories that have been put forward to explain the steps that are involved in the development of a venous ulcer and are described below.

Arteriovenous fistulas

This is the oldest microcirculatory theory of venous ulceration which suggests that small arteriovenous fistulas transmit arterial pressure to the venous circulation with resulting

increases in vascular permeability that ultimately lead to abnormalities of oxygen and nutrient delivery to the skin.

However, studies using radio-labelled albumin to measure shunt volumes in lower extremities of patients with primary varicose veins versus controls showed no detectable differences between the two groups (Lindemayer et al, 1972).

Currently, modern data do not support this theory as a cause of venous ulceration.

Fibrin cuff theory

It was proposed that the increased pericapillary fibrin cuffs that surround perimalleolar cutaneous capillaries in chronic venous insufficiency patients prevent adequate diffusion of oxygen and other nutrients into the surrounding tissues. This is the fibrin cuff theory of venous ulcer pathogenesis proposed by Browse and Burnand (Browse et al, 1982). They speculated that venous hypertension led to widening of endothelial gap junctions with subsequent extravasation of fibrinogen leading to the development of fibrin cuffs which then act as a barrier to oxygen diffusion and nutrient blood flow and cause epidermal cell death.

White cell trapping theory

This is the most recent theory concerning the pathogenesis of venous ulceration and it focuses on the adverse effects of white blood cells trapped within the skin microcirculation. Subsequent observations of decreased circulating leukocytes in blood samples obtained from the long saphenous veins in patients with chronic venous insufficiency led Coleridge Smith et al to propose the white cell trapping theory (Coleridge Smith et al, 1988). This theory states that circulating neutrophils are trapped in the venous microcirculation secondary to venous hypertension. The subsequent sluggish capillary blood flow leads to neutrophil activation. Neutrophil activation leads to degranulation of toxic metabolites with subsequent endothelial cell and skin damage. There are now proposed treatment strategies based on presumed activation of proteolytic enzymes released by entrapped white cells.

1.10 Summary

Chronic venous disease of the lower limbs has been shown to be a major problem in the western world with severe economic consequences. Its clinical sequelae range from oedema, haemosiderosis and pigmentation, to gross lipodermatosclerosis and venous

A biomechanical hypothesis

In the early 1970s, Chant et al carried out a series of experiments in which the clearance of subcutaneously injected sodium-24 was measured. It was found that there was an almost perfect inverse relation between sodium-24 clearance and changes in venous pressure with posture.

Tanner et al in 1989 investigated the hyperaemic response following 3 minutes of ischaemia in the microcirculation in patients with venous leg ulcers using laser Doppler fluxmetry. They concluded that there was a reduced hyperaemic response in patients with venous ulcers, possibly as a result of oedema acting as a mechanical barrier to vasodilation.

Chant postulated a mechanical cause for ulceration based on the elevated tissue pressure in a mechanically disadvantaged area, the gaiter area, of the lower limb (Chant et al, 1990). He proposed two hypotheses, firstly, that the presence of oedema in patients with venous disease results in a rise in tissue pressure and this causes the postural changes in the circulation to be modified. Secondly, the raised vascular and tissue pressures in non-compliant areas, the gaiter area (high bone/soft tissue ratio) result in continuously high skin tension leading to ischaemia of the skin which then causes ulceration. High tissue pressure was seen as a prime determinant of postural responses as well as the prime factor in the initiation of venous ulceration.

Biomechanical studies (Mourad et al, 1988) using an extensometer, on the skin of patients with venous ulceration and in patients with lymphoedema, showed that the characteristics of skin in lymphoedema differed markedly from controls and those with venous ulcers. Patients with chronic venous disease leading to leg ulceration have greatly reduced elasticity of their skin. Chant (1992) explains that a rise of pressure in the inelastic skin of patients with venous disease leads to a much greater increase in tissue tension than in patients with lymphoedema in whom the skin elasticity is not reduced. The increase in skin tension in patients with venous disease is the factor which leads to ischaemia of the skin leading to ulceration.

This hypothesis explains why venous ulceration occurs in the ankle region, but not the biological events which lead to loss of skin compliance in patients with chronic venous disease.

References

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ulceration with the gaiter area as the site most commonly affected in the lower limb. The extent and severity of the disease can be assessed by clinical and physiological methods described earlier. A vast majority of patients show a wide spectrum of skin changes of varying severity which precedes venous ulceration.

At present, there is no standardised objective method of assessing the mechanical properties and degree of skin change in these patients, so that the response to treatment can be objectively monitored.

1.11 Hypotheses

- The development of lipodermatosclerosis precedes the appearance of venous ulcers in every case. This can be identified easily by clinical examination because of the changes in mechanical properties of the skin. The changes in the skin can be measured using a mechanical sensor in the form of a tissue tonometer.
- Patients with the more severe forms of venous skin changes (LDS, ulceration) according to the CEAP classification have the greatest disturbances in tissue properties as demonstrated by tissue tonometry.
- Patients with LDS when assessed clinically and divided into those more severely affected and less severely affected show corresponding measurements of tissue tonometry.
- Treatment of patients using compression hosiery which is well known to be effective in the management of LDS. The effect of this type of management is measurable when assessed by tissue tonometry.
- Ultrasound imaging of the skin and subcutaneous tissues in patients with venous disease reflects the same changes shown by tissue tonometry.
- Duplex ultrasonography is widely used in the assessment of venous disease. Air plethysmography is required to achieve a quantitative assessment of the severity of the venous pump impairment in patients with venous disease.
- The severity of the venous pump damage predicts the severity of the skin damage in particular patients, as reflected by tissue tonometry.

1.12 Aims

- To standardise the calibration, repeatability and reproducibility of the methodology of the tissue tonometry measurements

- To investigate whether the tonometer can differentiate between the mechanical properties of the skin in normal controls and in patients with lower limb chronic venous disease.
- To use an *in vitro* model, the sponge-in-glycerol model, to obtain displacement versus time curves so as to apply a simple visco-elastic mathematical model, the Kelvin-Hooke model, on the curves obtained to calculate the spring and dashpot constants to explain its mechanical behaviour.
- To compare the clinical assessment of the severity of liposclerotic skin changes made by an experienced vascular surgeon using the CEAP method with the tissue tonometry parameters of skin compliance in patients with bilateral LDS.
- To measure skin and subcutaneous layer thickness in control and patient groups with lower limb venous disease using a 7.5 MHz transducer and correlate this with displacement versus time curves obtained using the tissue tonometer.
- To measure the effects of compression stocking treatment for 6 weeks on the mechanical properties of the skin of patients with lower limb chronic venous disease using a tissue tonometer and to compare this with limb volume changes after compression stocking wear.
- To assess the inter-variability between examiners and the ease of use of the CEAP method of classification and scoring of lower limb venous disease.
- To correlate duplex-derived parameters of lower limb venous reflux with air plethysmography measurements and the clinical severity of venous disease classified by the CEAP method with tissue tonometry.

SECTION II

Protocols & Methodologies

2.1 Protocol for quantifying lower limb venous reflux using duplex ultrasound scanning

In this thesis, all colour duplex ultrasonic mode imaging, B-mode (brightness mode) imaging and spectral Doppler mode measurements were performed by me using the Acuson 128XP/10v (Acuson, Mountain View, California, USA) computed sonography system with ART (acoustic response technology) using a 5- or 7.5- MHz linear array transducer.

2.1.1 Methodology

Patient preparation

The ultrasound examination was done with the patient in the standing position so that the veins were under hydrostatic pressure, as in pathological conditions. The limb to be examined was externally rotated and completely relaxed and non-weight bearing with all the weight transferred to the contralateral limb.

Venous segments that were as close to the gaiter area as possible without interfering with the cuff positioned on the calf were examined: the SFV in the distal thigh, distal popliteal vein segment, proximal PTV segments, LSV at knee level and proximal SSV segment. Measurements at the valve cusps were avoided due to the presence of turbulent flow and all measurements were carried out on segments just above or below the cusp. In the quantification of blood volume flow within a vein, the size of the pulsed Doppler gate insonated the entire vessel in order to account for all the blood cells which travel at different velocities with the slowest velocities present towards the vessel walls and the fastest present in the centre of the vessel (see figure 1.4.3.1).

The method used below has been previously described by Bemmelen et al in the quantification of venous reflux (van Bemmelen et al, 1989). The long saphenous vein and superficial femoral vein were examined with the patient in the standing position and facing the examiner, with the leg to be examined slightly flexed and externally rotated with the body weight supported by the contralateral limb. The sapheno-popliteal junction, short saphenous vein and popliteal vein are examined with the patient standing and facing away from the examiner with the knee on the side being examined slightly flexed so as to relax the popliteal fossa and the weight distributed on the contralateral limb. A pneumatic calf cuff with a width of 12cm that could be rapidly inflated and deflated automatically to a pressure of 120mmHg was placed around the calf and used to induce reverse flow. A standardised measuring method was adopted throughout in all

the vein segments examined after augmentation of blood flow by the standardised cuff inflation method in the calf. A standardised rapid cuff inflation and deflation unit constructed by the author (Middlesex Hospital Vascular Laboratory, 1995) with a calf cuff width of 12cm was used to augment flow in the vessels (see section 2.7). The cuffs were inflated for approximately 3 seconds with the deflation requiring only 0.3seconds. Initially a B-mode cross-sectional 2D image of the vein segment of interest was obtained after insonation of the vessel with plenty of scanning gel applied between the transducer and the skin to avoid distortion of the underlying superficial vessels that may be compressed with the transducer. The system software cursors were positioned to measure the vessel diameter. A longitudinal B-mode image of the same vessel at the same site as the diameter measurement is used to obtain a frozen spectral Doppler display of flow in the vessel after calf compression and release to demonstrate both the antegrade and retrograde flow.

2.1.2 Data Analysis

To calculate reflux or retrograde volume blood flow

The formula used to calculate the reflux volume blood flow is

$$\mathbf{Q = TAV \times area \times \Delta T}$$

where, Q = reflux volume flow (ml), TAV = time averaged velocity (metres / sec), Area = cross-sectional area (mm^2) at the Doppler sample gate and ΔT = a multiplier to express flow within the duration of reflux (seconds).

The formulae ($\pi D^2/4$ or πr^2) where D is the cross-sectional diameter of the vein in mm obtained from the B-mode 2D imaging mode and r is the radius of the vein in mm is used to calculate the cross-sectional area of the vein and the TAV and ΔT can be calculated from the axial Doppler reflux velocity waveforms on the frozen spectral Doppler strip as described earlier.

To calculate the peak efflux and peak reflux velocity ratio

From the frozen spectral Doppler strip, the peak efflux or antegrade flow velocity is measured by placing a single calliper marker to measure the angle corrected velocity parameter of the Doppler signal at that specific point in time. The same is repeated to measure the peak reflux or retrograde flow velocity (see figures 2.1.1 and 2.1.2). The peak efflux and peak reflux velocity ratio is calculated by dividing the peak efflux

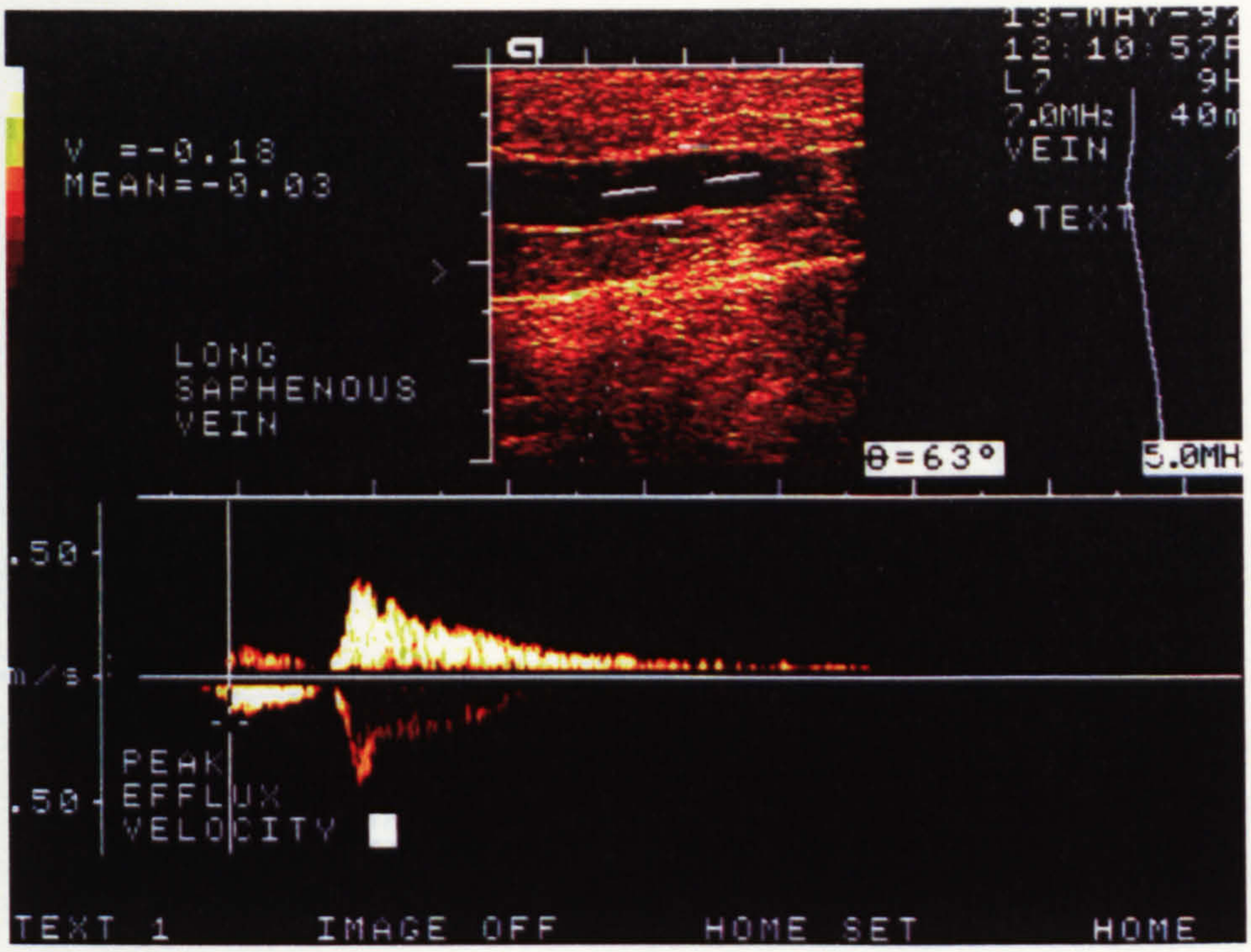


Figure 2.1.1 Spectral Doppler analysis used to calculate the peak efflux velocity by placing a cursor at the highest point of the antegrade waveform.

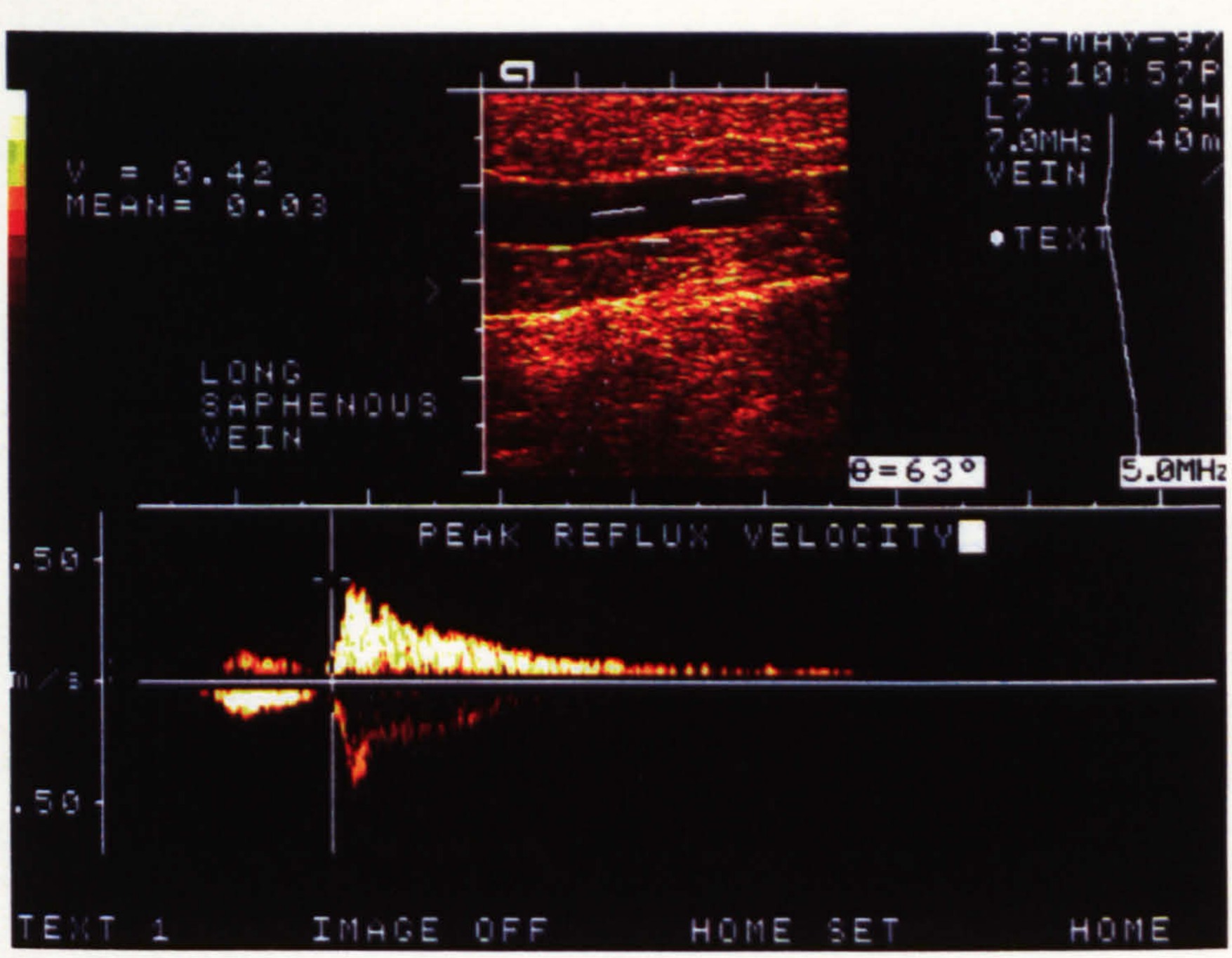


Figure 2.1.2 Spectral Doppler analysis used to calculate the peak reflux velocity by placing a cursor at the highest point of the retrograde waveform.

velocity value by the peak reflux velocity value (Pk efflux vel (m/s) / Pk reflux vel (m/s)).

To calculate the time parameters and TAV parameter

The time parameters and TAV measurements are calculated by the ACUSON 128 system computer software. The duration of the efflux flow and reflux flow in seconds can also be obtained by placing the calliper markers at the start and end of the waveform. The efflux and reflux time ratio is calculated by dividing the efflux time value by the reflux time value (efflux time (secs) / reflux time (secs)).

The valve closing times, VCT, which is the reflux time parameter calculated from the spectral Doppler trace, were calculated for all the incompetent vein segments and added up to give the total valve closing times, tVCT, in seconds, for each patient. The relevant segments of veins in the normal controls were measured for comparison.

To calculate area measurements

The area measurements in cm^2 of the efflux flow and reflux flow are traced with a sonic digitiser pen (Science Accessories Corporation model GP-7) on the frozen spectral Doppler display to calculate the reflux index (efflux area (cm^2) / reflux area (cm^2)).

The Doppler Efficiency Index (EI d) is calculated as follows:

$$\textbf{EI d} = \frac{\textbf{AA - AR x 100}}{\textbf{AA}}$$

where AA represents the area of the efflux or antegrade flow and AR represents the area of the reflux or retrograde flow (figure 2.1.3).

Measuring height, weight, Body Mass Index and Body Surface Area.

The heights and weights of the subjects were measured using a tape measure and a weighing scale.

The body mass index (Kg/m^2) which is (weight (Kg)/ $height^2$ (m)) and the body surface area (m^2) which is calculated by the ACUSON 128 system were recorded for each subject.

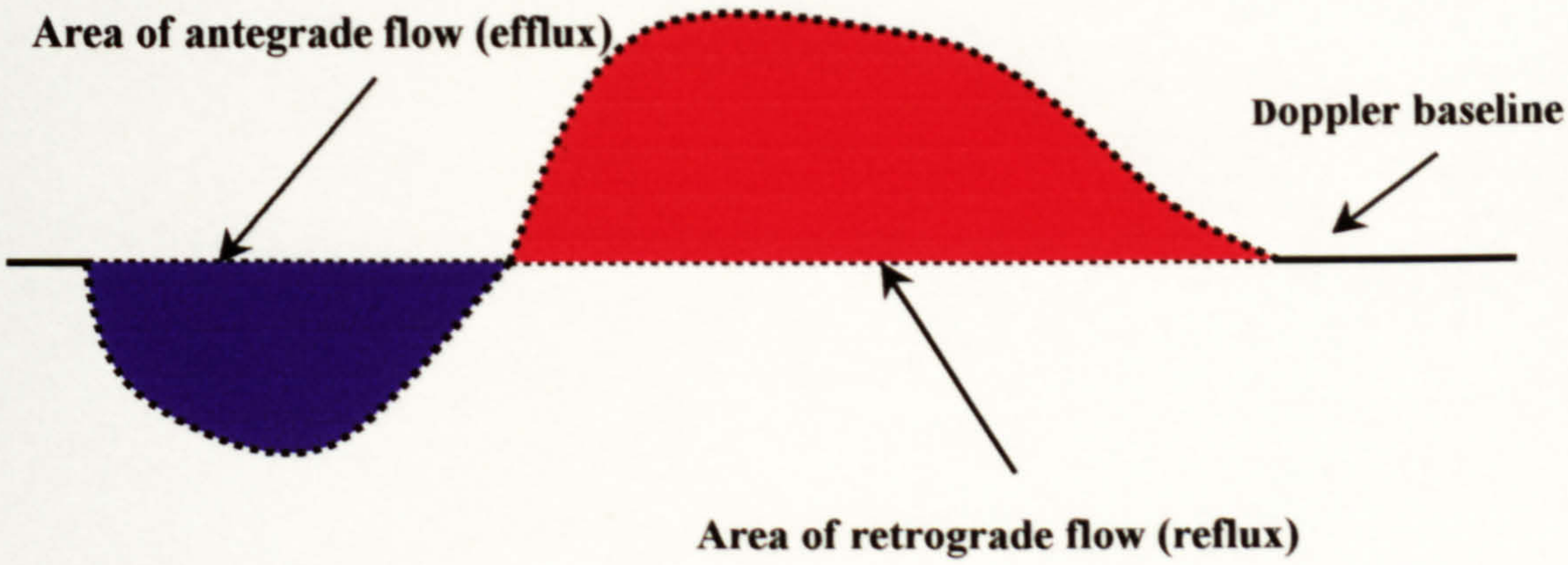


Figure 2.1.3 Schematic diagram of a spectral Doppler trace obtained after flow augmentation in a vein. The area of antegrade flow (efflux) after compression of the calf muscles indicated by the blue shaded area which has been traced over using a digitiser pen and the area of retrograde flow (reflux) after release of the calf muscles indicated by the red shaded area (reflux) which has also been traced over to give an area measurement. The areas measured above and below the Doppler baseline are used to calculate the ratio of efflux and reflux flow to get the reflux index.

2.2 Protocol for air plethysmography measurements of venous function and arterial perfusion.

The air plethysmograph used throughout in this thesis is the APG-1000C (ACI Medical inc., Sun Valley, California). It is fully computerised with all data analysis performed on the computer screen by means of cursors with a thorough and easy guide on where and how to position the cursors for calculation of the parameters (APG® Air-Plethysmograph, Models APG-1000C and APG-1000CP, Instruction and Service Manual, Version 2.11).

Methodology

Patient preparation

The subject was prepared in the same way for all the parameters to be measured with calibration performed prior to the start of each parameter and in the order described below. The lower limb sensing cuff comes in two sizes, a 14 inch cuff and a 12 inch cuff. The largest sensing cuff that would fit the subject's leg but not interfere with the foam block or the back of the most distal thigh with the leg slightly bent was selected. When applying the sensing cuff to the calf, the zipper was located on the anterior surface. The tubing that leads from the cuff to the main control unit were taped to the thigh. The subject's arms were placed by their sides and not rested on the abdomen to avoid pressure on the abdominal veins.

The subjects lay on the examination table with the heel of the limb to be examined elevated on a foam support, 15cm high, with the leg externally rotated and the knee slightly bent. In this thesis, I have standardised the knee bend by using a boomerang (Wagga Wagga, Australia) with a fixed 145 degree angle in all the subjects by placing the boomerang behind the knee during subject preparation to get a standardised knee-bend angle.After setting the 'bias pressure' to 6 mmHg and ensuring that the calibration syringe was completely withdrawn, the sensing cuff, in position as described above, was inflated to the selected pressure which was actively maintained by the internal pump or bleed-off valve as necessary. For all air plethysmography tests performed in this thesis a 10 minute stabilisation period was allowed for each subject to ensure temperature equilibration prior to calibration and all the tests were performed at the same time of day (between 14.00 and 15.00 hours) for all subjects. The machine was re-calibrated prior to each measurement made in every subject. Steps were also taken to ensure that the

subject being examined was not in direct sunlight or near a radiator to prevent false high readings in cuff pressure as a result of thermal heating of the air in the sensing cuff.

Calibration

Calibration was performed in two parts. Firstly, the calibration syringe was pulled out to its stop position with the sensing cuff at a stabilised bias pressure of 6 mmHg on the subject and the pressure saved. One hundred ml of air was slowly injected into the cuff from the calibration syringe and after allowing 5 seconds for the pressure to reach a steady state it was saved to store the cuff pressure. The calibration syringe was then withdrawn back to its 100 ml mark and the calibration signals sent to the computer. Calibration must be performed whenever the sensing cuff is applied to a limb and for studies done in this thesis, calibration was performed prior to each parameter measurement. The methodology for all the APG parameters recorded are described below in the order performed: outflow fraction; resting arterial inflow; venous filling index; ejection fraction and residual fraction. This is shown diagramatically with the typical traces obtained in figure 2.2.1.

2.2.1 Methodology for Outflow Fraction (OF) testing for chronic obstruction

When performing outflow studies, the thigh occlusion cuff (10 cm wide) was applied as far proximally as possible with the rapid deflation device attached to the cuff and the inflation bulb. With the subject properly prepared and the system calibrated as described above, the thigh cuff was inflated to 70 mmHg and the veins allowed to fill completely by observing the volume indicator on the screen. After applying finger pressure to the long saphenous vein or any other visible thigh veins, which were identified with duplex scanning, the cuff was rapidly deflated to drop the thigh cuff pressure. This would result in a decrease in calf volume as blood exits past the thigh cuff. If the outflow fraction calculated as described below, is greater than 35% then a second outflow test without superficial venous occlusion is not necessary.

Data analysis

Analysis of the outflow tests are carried out by the computer's software by placing the cursors as described in the figure 2.2.2.

The outflow fraction (OF) was calculated by the formula:

$$\mathbf{OF\ (\%) = (V1/VC) \times 100}$$

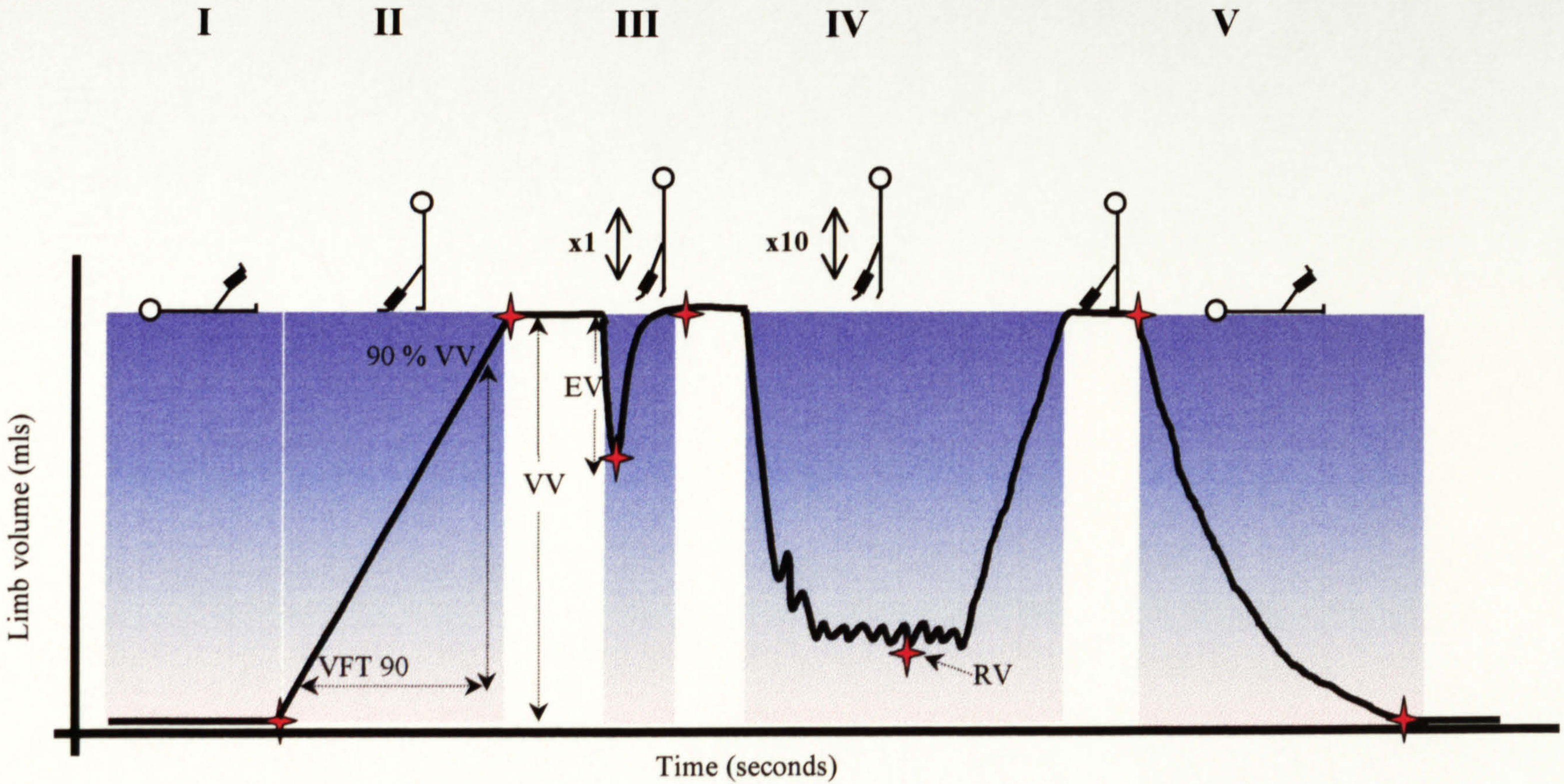


Figure 2.2.1 Typical traces of limb volume changes recorded during a sequence of postural changes and exercise: (I) subject supine with leg elevated 45 degrees; (II) subject standing with weight on non examined leg; (III) one tiptoe movement; (IV) ten tiptoe movements; (V) limb volume returning to baseline.

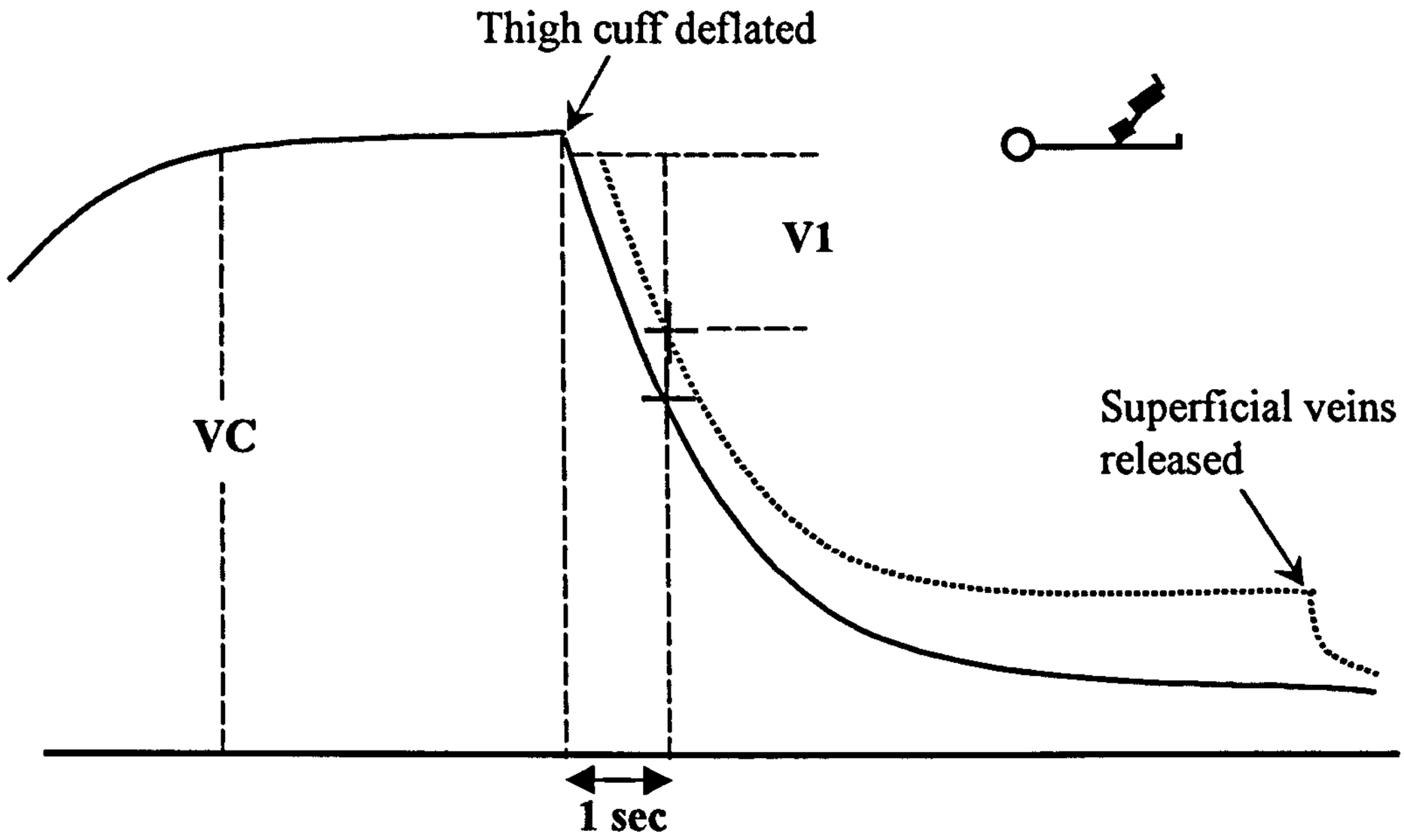


Figure 2.2.2 Measurement of outflow fraction. The thigh cuff is inflated to allow veins to fill and with the superficial veins occluded the thigh cuffs are then deflated. The 1 second outflow is measured.

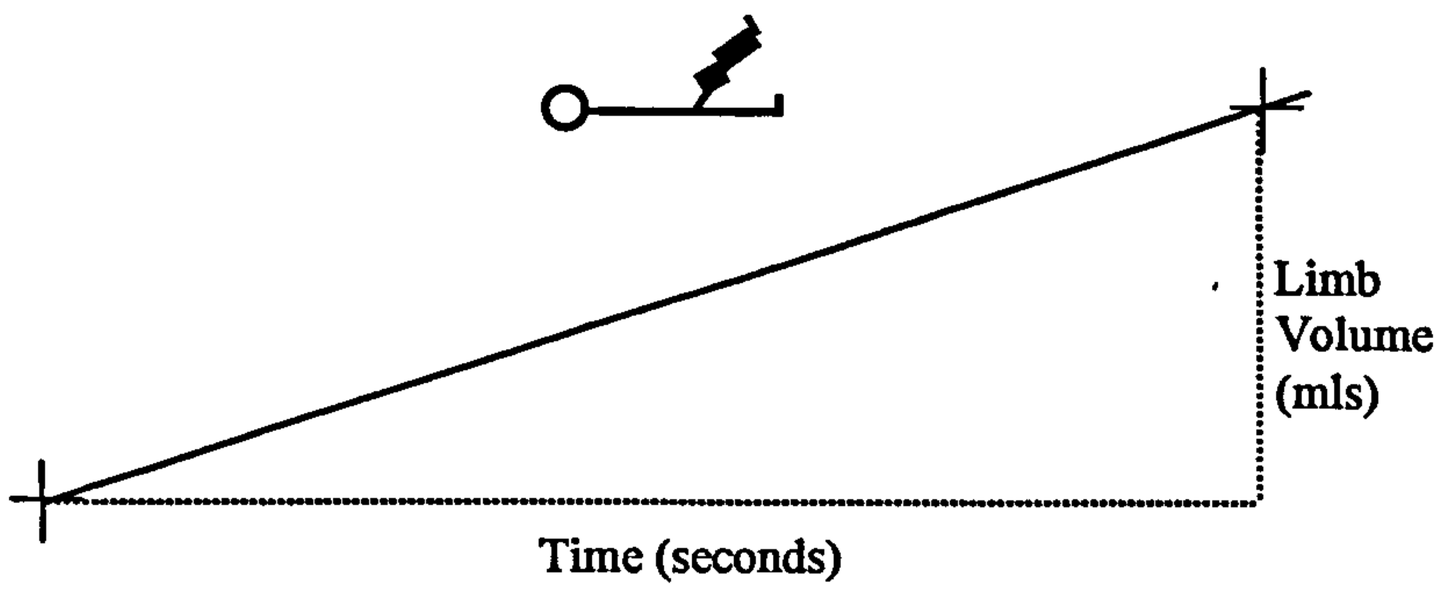


Figure 2.2.3 Measurement of arterial inflow (AI). The knee tourniquet is inflated and the slope of the line is the arterial inflow rate in ml/min.

where, (V1) is the volume of blood that exits the calf in the first second and (VC) is the venous capacitance.

2.2.2 Methodology for Arterial inflow (AI) measurement

The subject was prepared as described in section 2.2. The 10cm cuff was placed just above the knee ensuring that it was not interfering with the sensing cuff. The rapid deflation device was attached to the cuff and to the inflation bulb. After calibration, the above knee cuff was rapidly inflated to 70 mmHg which resulted in an initial steep volume increase followed by a less steep but mostly linear volume increase. The initial steep rise is an artefact that arises as a result of inflation of the cuff and the measurement starts after this artefact on the linear portion of the trace for 25 seconds. The arterial inflow (AI) was calculated by the system's software in ml/minute by the following formula:

AI = 3 x ml increase in 20 seconds.

Data analysis

Analysis of the outflow tests were carried out by the computer's software in mls/minute by placing the cursors as described in figure 2.2.3.

2.2.3 Methodology for Venous reflux testing (Venous Filling Index)

The subject was prepared and the system calibrated as described in section 2.2. The limb to be examined was elevated to a 45 degree angle by holding the heel of the foot and avoiding touching the sensing cuff. The subject was instructed to keep the knee slightly bent and to allow the full weight of their leg to be supported by the examiner.

The recording was started and the trace observed as it dropped to a baseline. At this point, the calf had reached its zero functional venous volume as the blood was drained towards the heart. The subject was guided off the examination couch slowly whilst ensuring that the sensing cuff was not touched at any time. The subject was asked to stand still with no weight on the test limb that had the sensing cuff and with both feet side by side. Weight must be supported by the untested limb with the subject maintaining balance by holding onto the walker support. An increase in volume would be observed on the screen and when this volume had reached a plateau by observing both the trace and the volume indicator on the screen, the recording was stopped to give the maximum venous volume.

Data analysis

To calculate the venous filling index (VFI), the zero point which is the lowest point just as the trace starts to rise and is zero for volume and for the time of the start of the increase in the calf volume from standing up was selected on the screen. The maximum venous volume was also selected and is the point when the veins had completely filled after standing up. The VFI is calculated by the system's software in ml/sec by the formula, as shown in figure 2.2.1:

VFI = 90%VV/VFT90

This increase represented the functional venous volume (VV) volume which is a result of filling of the veins as the subject moved from the supine to the upright position due to arterial inflow and venous reflux if present. The time taken to reach 90% of filling was defined as the venous filling time 90 (VFT90).

2.2.4 Methodology for Calf muscle pump testing for Ejection Fraction (EF)

Immediately following the VFI test for reflux and with the subject remaining standing with no weight on the tested limb and the veins filled to their maximum venous volume, the EF test was selected on the screen and the subject asked to perform one tip-toe movement following these strict guidelines. Full body weight was placed equally on both legs side by side and the subject asked to go up on tip toes as high as possible and hold for about two to three seconds and then to return to the standing position with the weight supported equally by both legs. Then the subject was asked to return to the initial position with no weight on the tested leg and the knee slightly bent with both feet side by side. The result of calf muscle contraction compressing the deep veins will appear as a decrease in volume on the trace. After the veins had refilled, the subject was asked to repeat the single tip-toe movement. This procedure was repeated until the subject understood the exercise and repeatable calf ejections were produced. The recording was then stopped and the trace that represented the subject's best effort was used for analysis.

Data analysis

To calculate the ejection fraction (EF) the lowest point of the ejected curve and the refill volume was selected on the computer screen and the traces analysed by the system's software to calculate the ejection fraction.

The % ejection fraction (EF) was calculated by the formula as shown in figure 2.2.1:

$$\mathbf{EF(\%) = (EV / VV) \times 100}$$

where, EV is the ejected volume and VV is the venous volume found in the VFI (reflux) test.

2.2.5 Methodology for Residual Volume Fraction (RVF) testing

After completing the ejection fraction test with the subject standing with no weight on the tested limb and with the veins full and a volume plateau reached, the RVF test procedure can be started. This time, the subject was asked to perform ten tip-toe movements approximately one per second following the same protocol described above for the EF test. After this exercise, the subject allowed the veins to refill by standing again without weight on the tested limb. Once refilled, the subject was asked to return to the examination table and elevate the limb to approximately 45 degrees. The subject was reminded that the knee should be kept slightly bent and that the full weight of the limb be supported by the examiner's hand placed under the heel. Care was taken for the sensing cuff not to be touched at any time during the manoeuvres. When the trace reached a new baseline representing venous emptying, the recording was stopped.

Data analysis

The RVF test was analysed by selecting the lowest tip-toe point and the ending baseline and the traces are analysed by the system's software to calculate the residual volume fraction. It was calculated from the formula as shown in figure 2.2.1:

$$\mathbf{RVF\ (\%) = (RV/VV) \times 100}$$

where RV is the residual volume and VV is the venous volume.

An important point to remember while calculating the RVF is the ending baseline. The difference between the beginning baseline at the start of the VFI test and the ending baseline in the RVF test should be less than 20%. If they were significantly different (20% or more), the beginning baseline should be used as the ending baseline.

2.3 Protocol for Venous reflux test by photoplethysmography (ppg).

The model PPG13 Vasculab® Photoplethysmograph (PPG) (Medasonics, Mountain View, California) was used in this thesis. It serves as a preamplifier and power supply and was used with the model PH77 PHOTOPULSE® photoplethysmograph sensor

Methodology

The subject was examined in the sitting position with the lower limbs dependent and with the transducers attached using a double-sided tape 5cm proximal to the medial malleolus. A stable trace was established, and the subject was asked to perform a series of 10 dorsiflexions and plantarflexions at the ankle to activate the calf muscle pump. This produced an emptying phase in which the falling content of haemoglobin resulted in increased amounts of light reaching the photodetector. The subject was then asked to rest and the refilling phase recorded. If the test showed an abnormal response, a tourniquet cuff (5 cm dia) was placed above the knee and inflated to about 80-110mmHg (depending on the size of the thigh) to occlude the superficial veins in the thigh and the test repeated as described above. If the test still showed an abnormal response, it is repeated with below knee cuffs inflated to 40-60 mmHg to occlude the calf veins.

Data Analysis

The time taken for the trace to return to the baseline level was referred to as the PPG refilling time. It was measured by placing the start cursor at the end of the exercise phase and the end cursor at the end of the refilling phase where the trace started to level off. In normal subjects, this refilling time is greater than 20 seconds (figure 2.3.1) whereas abnormal subjects with incompetent veins will have refilling times of less than 20 seconds (figure 2.3.2).

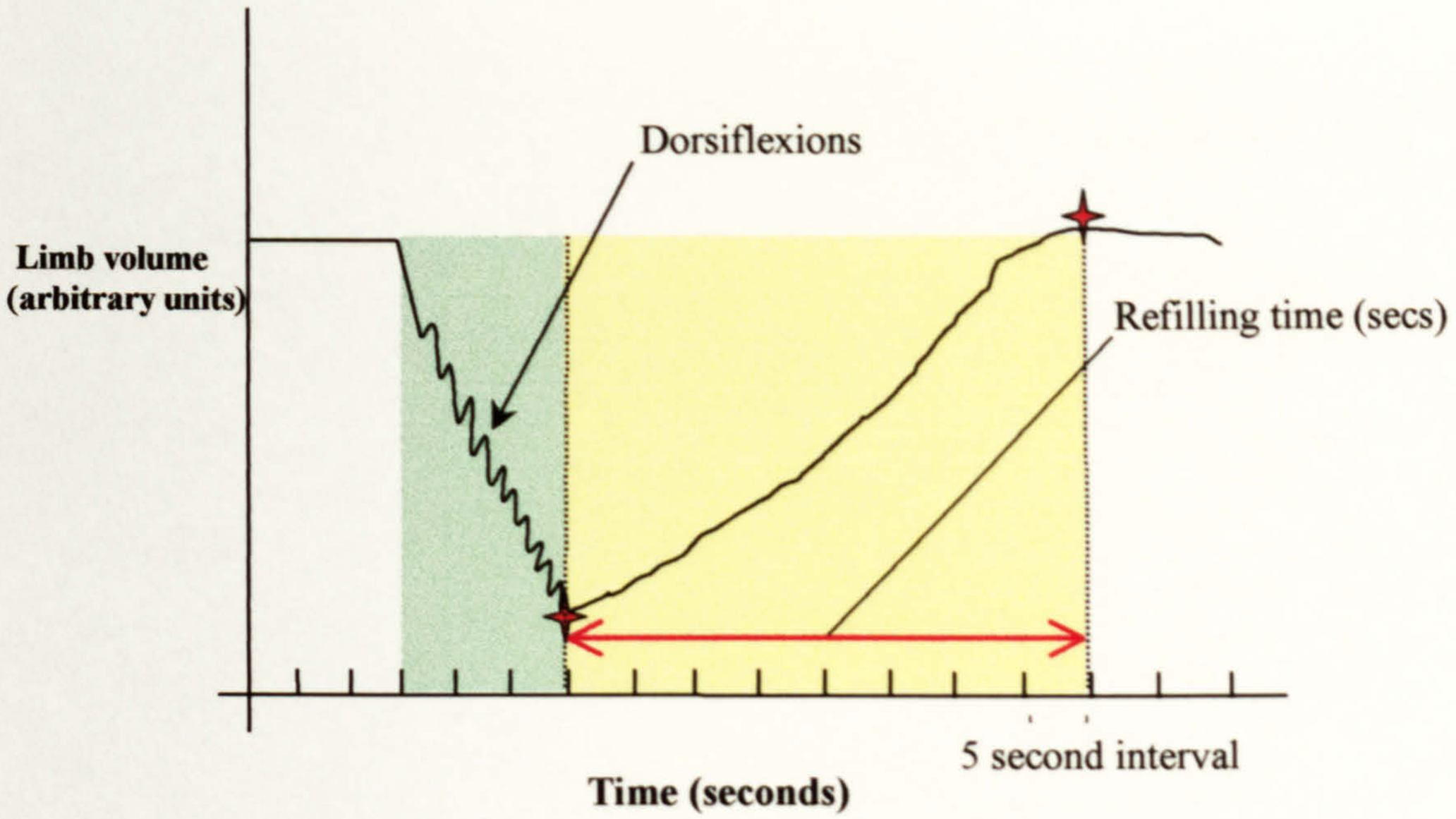


Figure 2.3.1 Normal PPG trace with the refilling times (the start and finish times shown by the red stars) lasting longer than 20 seconds during the recovery period (yellow-shaded area) after exercise by dorsiflexions of the ankles (green-shaded area).

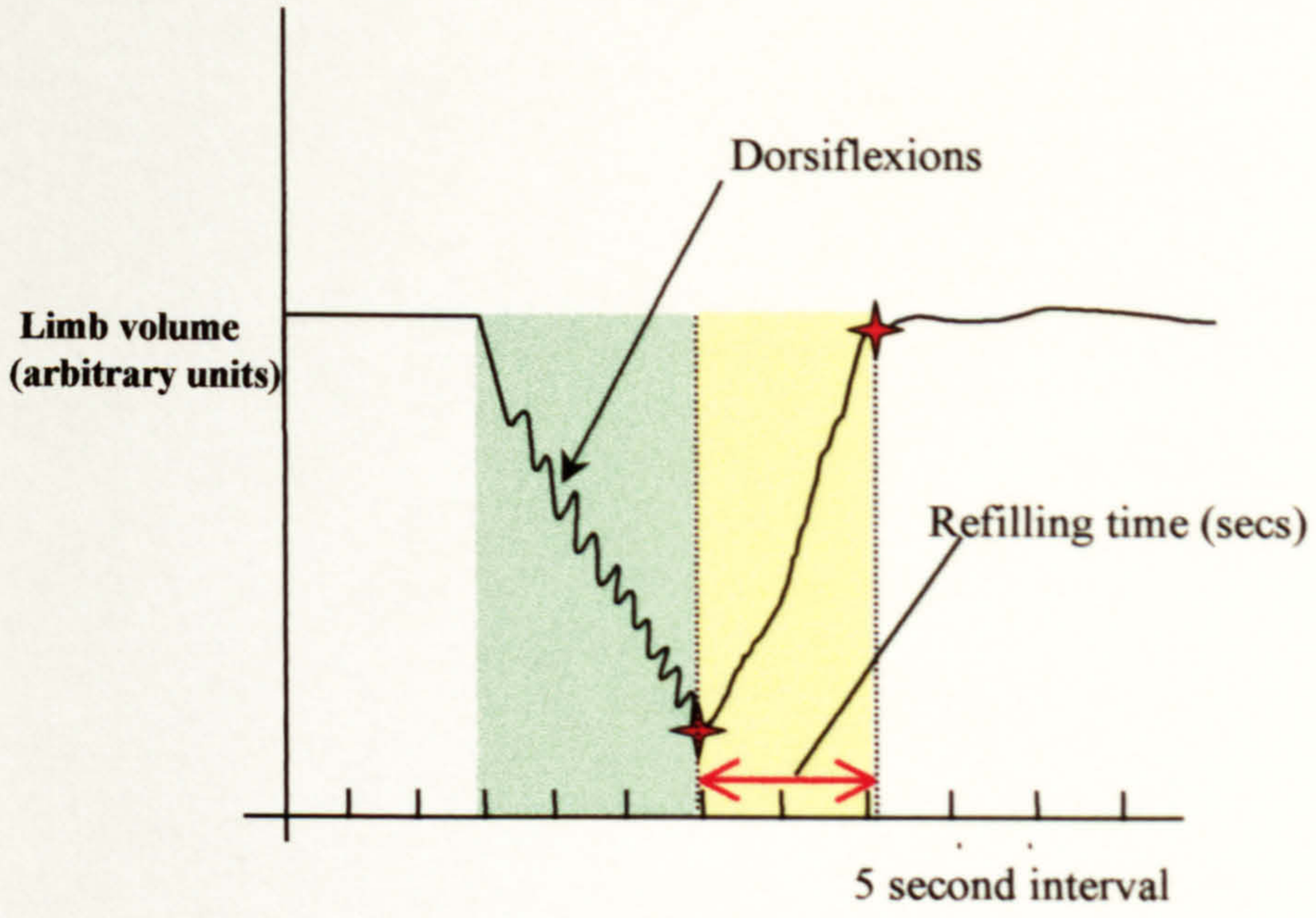


Figure 2.3.2 Abnormal PPG trace with the refilling times (the start and finish times shown by the red stars) lasting less than 20 seconds during the recovery period (yellow-shaded area) after exercise by dorsiflexions of the ankles (green-shaded area).

2.4 Protocol for measuring resting ankle brachial pressure indices (ABPI).

Subject preparation

The subject is examined in the supine position after lying relaxed in a comfortable ambient temperature room for 10 minutes.

Measuring arm pressures

A standard 12 inch sphygmomanometer cuff, or other suitable cuff (depending on size of limb) was placed around the arm just above the elbow. The brachial pulse was located with a hand-held bi-directional Doppler (Dopplex®, Huntleigh Healthcare) by positioning the 8MHz probe over the brachial pulse after applying gel and holding and manoeuvring the Doppler probe at a 45 degree angle between the forefinger and thumb until a good Doppler signal is obtained. The cuff was inflated until the Doppler signal disappeared and slowly deflated until the signal returned. The pressure at that point was noted as the brachial systolic pressure. The same procedure was repeated in the other arm.

Measuring ankle pressures

The sphygmomanometer cuff was placed around the leg just above the ankle and using the Doppler probe as described above, either the dorsalis pedis artery or posterior tibial artery pulse was located. The cuff was inflated until the Doppler signal disappeared and slowly deflated until it returned. The pressure at this point was noted as the ankle systolic pressure. Both pedal ankle systolic pressures were measured and recorded.

Data Analysis

To calculate the ankle brachial pressure index (ABPI), the ankle systolic pressure reading was divided by the brachial systolic pressure using the higher of the brachial pressures and the higher of the ankle systolic pressures in the calculation.

Normal ankle brachial pressure index is equal to or greater than 0.90.

2.5 Protocol for using the calibrated stand for site reproducibility

A specially designed calibrated stand by the author was used in site reproducibility for sequential measurements using tissue tonometry (section 2.8) and in the indirect method of measuring limb volume by the disc model method (see section 2.6)

It consisted of a calibrated column on a tripod stand which was fixed with a moveable disc and a marker pen attached that can be moved up and down the column and can be clamped on the calibrated column at a selected height (figure 2.5.1).

Subject preparation

The subject was investigated bare-footed on a flat surface with the marker pen positioned and parallel to the flat surface, checked with a spirit level, and pointing to the selected site of the lower limb

For subsequent visits, the reference points noted from the calibrated column, that is, the height of the marker and the positioning of the heel and toes in relation to the legs of the tripod, were used to re-locate the same sites previously used on the medial and lateral aspects of the subject's limb.

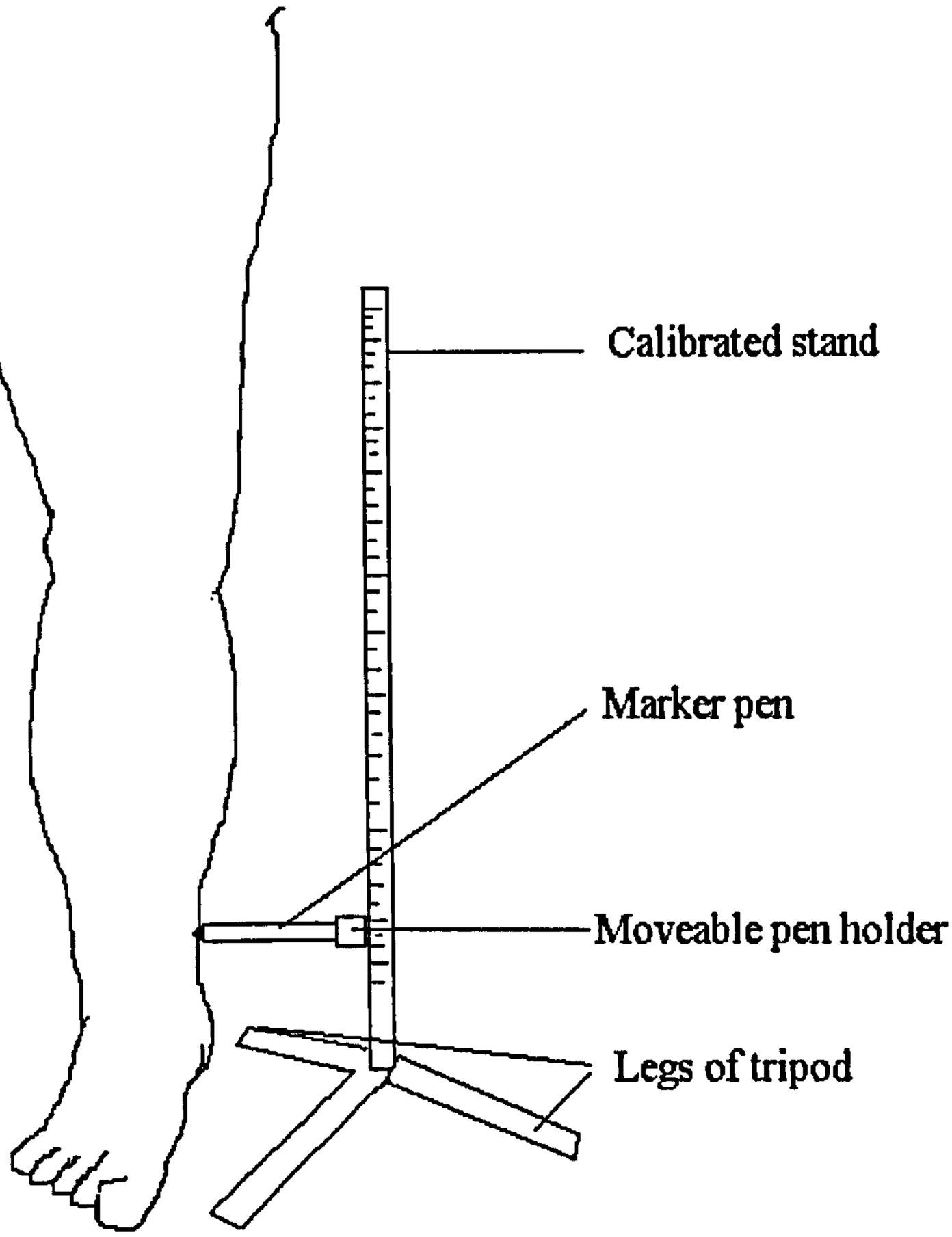


Figure 2.5.1 Schematic diagram of the stand with a fixed calibrated column and a moveable attachment with a pen holder that can be fixed to the calibrated column. The legs of the tripod can be used as markers to the body landmarks such as the heel and big toe to ensure site reproducibility on sequential visits.

2.6 Protocol for the indirect method of quantification of lower limb volume

The disc model method (Kaulesar-Sukul et al, 1993) was used to quantify the lower limb volume using a non-stretch tape measure and a calibrated stand designed by the author in section 2.5).

Patient preparation

The subject was investigated bare-footed on a flat surface with the calibrated stand and fixed marker pen which is parallel to the flat surface, checked with a spirit level, and pointing to the medial aspect of the lower limb.

Disc model method

The starting height corresponding to just above the medial malleolus was noted on the calibrated column. A mark was then placed on the skin. The calibrated stand was then moved to the lateral aspect of the limb and a mark placed on the medial aspect at the same height. A non-stretch tape measure was used to measure the circumference of the disc by ensuring that the tape measure goes around the disc over both markers on the medial and lateral sides of the limb to avoid potential errors encountered like slipping when using a tape measure. The circumference of the disc was measured 3 times and an average value taken. In patients with varicose veins in the calf region, extreme care was taken with the tape measure to contour it around the calf so as not to compress the veins. The disc with marker attached was moved up the column by 3cm and the same repeated until all 10 discs had been marked for the Disc model method.

The volume of the limb was the sum total of the circumference of all the individual discs. For subsequent visits, the reference points noted from the calibrated column, that is, the height of the marker and the positioning of the heel and toes in relation to the legs of the tripod, were used to re-locate the same sites previously used on the medial and lateral aspects of the subject's limb.

2.7 Rapid inflation and deflation device

The rapid inflation and deflation cuff device was constructed by the author in our laboratory and is shown diagramatically below (figure 2.7.1).

It consisted of two solenoid valves, one connected to a compressor (JAVAC pump, JAVAC, U.K. Ltd.) which pumped air into the valve and a pressure manometer to monitor the pressure in the chamber. By means of a foot switch (Dewnaswitch, Skelmersdale, Lancs.) the air was allowed into the cuff through one of the solenoid valves and then rapidly deflated within 0.3 seconds by pressing the foot switch which allowed the air to leave rapidly through the other solenoid valve. The pressure in the cuff was monitored by a pressure manometer. The cuffs were inflated for approximately 3 seconds with the deflation requiring only 0.3seconds.

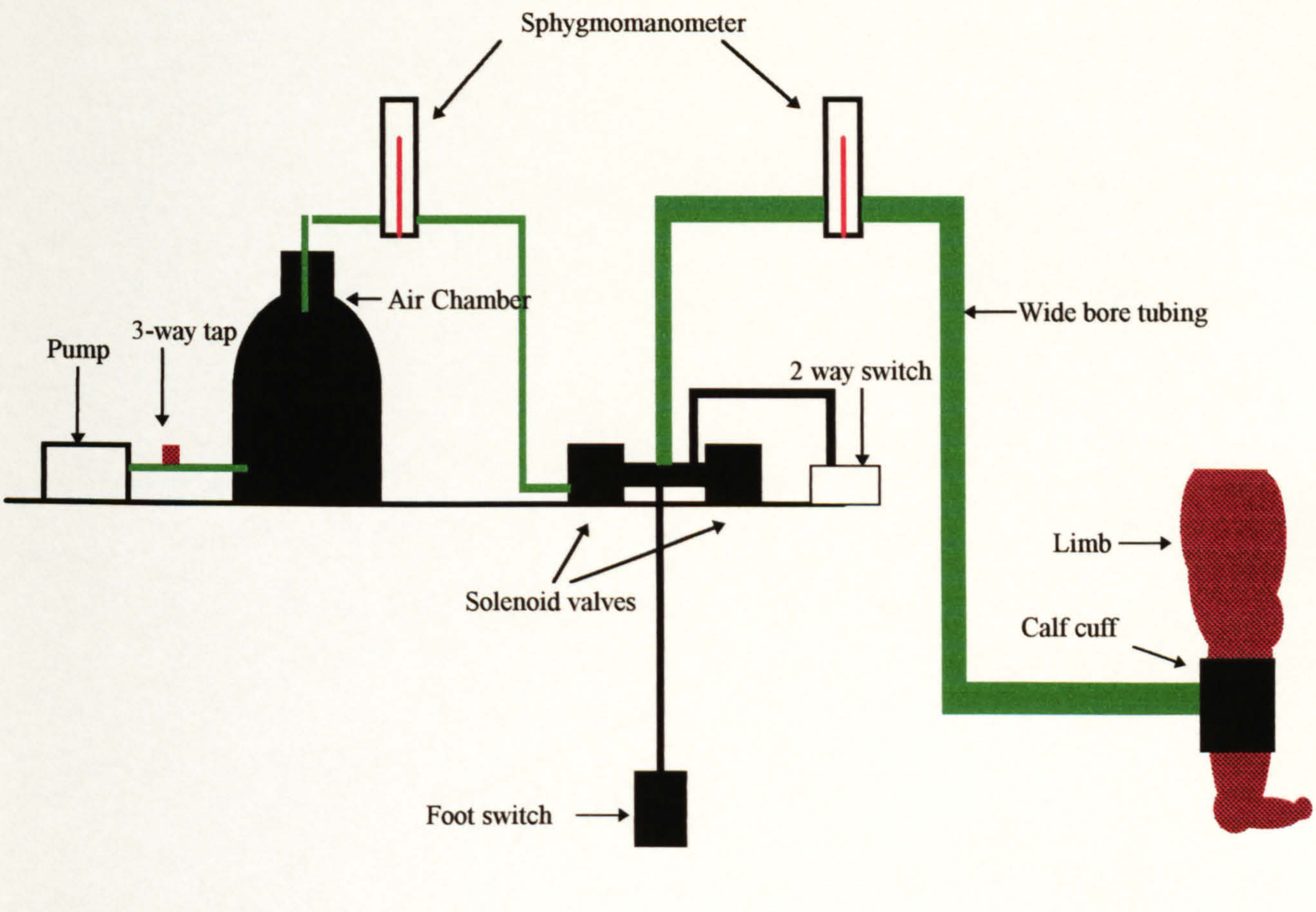


Figure 2.7.1. Schematic diagram of the rapid cuff inflation and cuff deflation device.

2.8 Protocol of calibration, reproducibility and methodology of tissue tonometry.

Calibration of the tissue tonometer

The tonometer was calibrated prior to and after each measurement. The calibration was performed by resting the tonometer on a level surface. A spirit level was placed on the casing that houses the sensing device to ensure that the cantilever bar was parallel to the level surface after both supports have been adjusted for height. The plunger with the selected weight attached was released unto a metal block of known thickness by means of the cable release mechanism and the plunger was allowed to drop to the level surface after removal of the metal block. The deflection on the screen was equivalent to the thickness of the metal block, and was used to calibrate subsequent measurements. A 3.3mm thick metal block, measured by a micrometer, was used in all studies.

Calibration check

The tonometer's calibration was checked using 4 brass discs of different thickness as measured by micrometer before and after 6 hours of recording on a 25% glycerol soaked sponge tissue (natural sponge cell, Boots plc). The calibration was performed by resting the tonometer on a level surface as described above. The 4mm diameter plunger with the selected 30g weight attached was released unto the brass disc of known thickness by means of the cable release mechanism and the plunger was allowed to drop to the level surface after removal of the disc. The deflection on the screen was equivalent to the thickness of the disc. The same procedure was repeated on the remaining three brass discs of varying thickness. The different thickness of the brass discs used as measured by a micrometer were 1.8mm, 2.6mm, 3.3mm and 5.6mm. Tonometry recordings on a 25% glycerol soaked sponge was performed for 6 hours and the calibration check repeated as described above.

Calibration reproducibility

A calibration reproducibility check was done before and after measurements to ensure that there was no significant drift in signal output between measurements.

The tonometer was calibrated before and after 5 minutes of recording on compression sponges soaked in 25% and 50% of glycerol diluted with water. A 4mm diameter plunger and a 30g weight attached was used in all the measurements. Thirty recordings were made on different sponges with fifteen sponges soaked in 25% glycerol and the

other fifteen sponges soaked in 50% glycerol. The measurements were made on two separate days with the gain altered on the second set of measurements (50% glycerol). This was to check whether altering the gain would affect the reproducibility of the calibration.

Patient methodology

Reproducibility of tissue tonometry sites

In all the tissue tonometry measurements performed to measure the mechanical properties of the skin, three different sites were measured on the medial aspect of the gaiter area to obtain a mean value. To ensure that identical sites were measured on the contralateral limb and in sequential measurements, a specially calibrated stand with a fixed marker pen, designed by the author, was used (see section 2.5). The subject was marked in the standing position and bare-footed on a flat surface, with the marker pen positioned, and parallel to the flat surface, checked with a spirit level, and pointing to the medial aspect of the lower limb. The first site was located 6cm above the medial malleolus, the second site was located 10cm above the medial malleolus and the third site was located 12cm above the medial malleolus. The starting height was noted on the calibrated column and the positioning of the small toe and the heel in relation to the legs of the tripod stand was also noted for future reference. A mark was then placed on the skin. For subsequent visits, the reference points noted from the calibrated column, that is, the height of the marker and the positioning of the heel and toes in relation to the legs of the tripod, was used to re-locate the same sites previously used (that is, same median line and same height above the medial malleolus) on the medial aspects of the subject's limb. With the three identical sites marked in both lower limbs using the reference points obtained from the calibrated stand, the subjects were then examined by tissue tonometry.

Tissue tonometry

All subjects were examined in the supine position with the test limb externally rotated and the knee slightly flexed so that the tissue tonometer could rest on the gaiter area as shown in figures 1.5.4, 1.5.5 and 1.5.6. After calibration, the tissue tonometer was positioned on the gaiter area of the lower extremity by contouring one end, the fixed contoured end, around the calf and the other end, which can be adjusted, resting on the medial malleolus or other suitable location. To improve equipment stability during

measurements, a Velcro cuff was placed around the calf so that the contoured end of the tonometer would rest on it to prevent it from sliding or falling off. The 4 mm dia plunger with the selected weight of 30g attached was positioned just above the skin area marked previously with the specially designed calibrated stand without causing any indentation.

The tonometer was allowed to stabilise on the subject's lower limb for 5 minutes to ensure that the ends of the tonometer would have settled on the leg with no further sinking into the tissues especially in cases of patients with oedematous ankles.

The 30g weight was released by means of a cable release mechanism and allowed to sink into the skin under gravity. The movement of the plunger was recorded for 5 minutes. After 5 minutes, the weight was released from the skin and the tonometer is re-calibrated. After re-calibrating the tonometer and allowing for the 5 minute stabilising period, the same procedure was repeated on the next selected site until all six sites have been recorded. The tonometer was re-calibrated after each measurement and if there was a significant difference between the pre- and post-calibration figures, the measurement was repeated.

Selection of attached weight and plunger diameter

Measurements were carried out on one site, located 10cm above the medial malleolus, on the medial aspect of the gaiter area of the lower limb of a normal control subject (CEAP classification, C_0) using different diameters of plungers and different weights attached.

The plunger with the selected diameter and the selected weight attached was positioned just above the area of skin of interest. After calibration, the weight was released and allowed to sink into the skin under gravity. The movement of the plunger was recorded for 5 minutes. The same procedure was repeated, allowing 120 minutes between measurements to allow the skin to return to its initial state, until all the different plunger diameters and attached weights listed below had been applied. The tonometer was calibrated prior to each measurement. The displacement versus time traces obtained were analysed using the computer software to calculate the distance and rate constant parameters in all three phases.

Data Analysis

The distance and rate constant parameters were calculated by using the specially written software as described in section 1.5.2.

The spring and dashpot constants were calculated from the Kelvin-Hooke model as described in section 1.5.3.

2.9 Protocol of cutaneous ultrasound measurement of skin and subcutaneous layer thickness.

The subject was examined in the supine position with the limb externally rotated.

A stand off pad, 1cm thick, was placed between the transducer and skin to avoid exerting pressure on the area of skin being examined.

A 7.5 MHz linear array transducer was used and held vertically to the skin surface at 90 degrees to obtain a B-mode image.

For correlation between the sonogram and tonometry readings, it was essential to mark the site of interest as both measurements were performed in exactly the same areas (Hoffmann et al, 1995 book). The mid-point level of the B-mode image obtained from the ultrasound machine on the medial aspect of the gaiter area was marked with a marker pen and the same site was used for the tissue tonometry measurement. The B-mode image obtained was analysed using the duplex scanner system's software by placing the cursors as shown in figure 2.9.1.

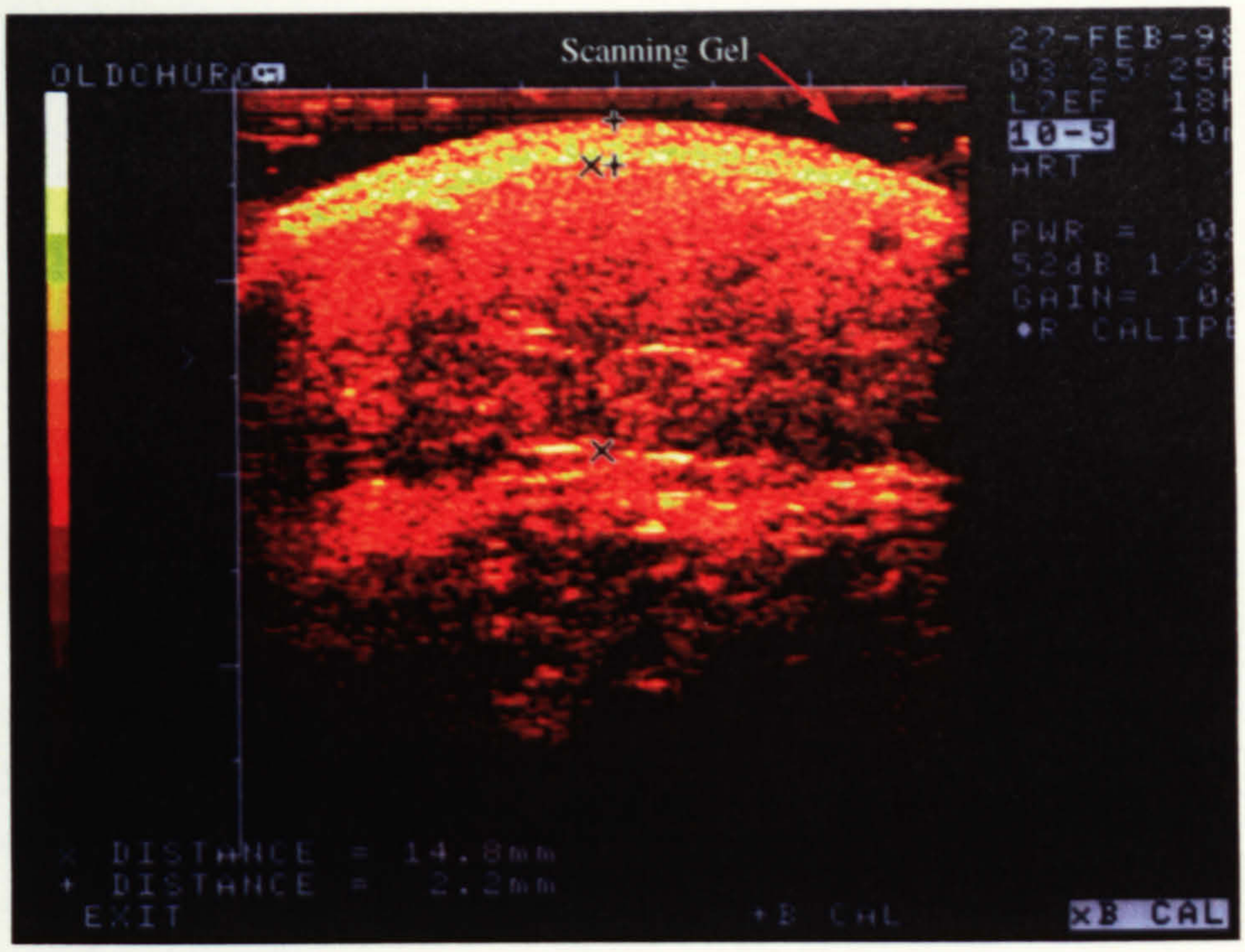


Figure 2.9.1 B-mode image with orange overlay to highlight the edges with cursors placed to measure skin thickness and subcutaneous layer thickness.

2.10 Modification of the CEAP clinical method of classification

In this thesis, I have standardised a new method of assessing the skin changes in patients with lower limb chronic venous disease. Preliminary studies with the tissue tonometer carried out on these patients during the initial stages of the thesis showed that there was a significant difference in the skin hardness between the group C_4 patients with pigmentation alone and no palpable evidence of induration as compared to other group C_4 patients with liposclerotic skin changes with palpable induration (see section 3.1; Study I). The difference was as much as three hundred percent which made it necessary to distinguish between these patients as the skin hardness measurement for the group C_4, which does not differentiate between the two, would have been diluted to give a much lower value when compared to groups C_5 and C_6 which have palpable evidence of induration. The group C_4 patients were then subdivided into C_{4a} and C_{4b} to differentiate between the skin hardness in the two groups as shown below and this was adapted throughout in this thesis, with the exception of Section 3.7; Study VII, that assessed the ease of use of the CEAP classification in its original format.

Therefore, the only change made to the CEAP method of classification and scoring of lower limb venous disease for use in this thesis was as a result of using tissue tonometry to analyse the mechanical properties of the skin in venous disease. The changes made are detailed in table 2.10.1 below:

Class 0:	No visible or palpable signs of venous disease.
Class 1:	Telangiectases or reticular veins.
Class 2:	Varicose veins.
Class 3:	Oedema.
Class 4a:	**Skin changes ascribed to venous disease (e.g. pigmentation with no palpable induration or previous ulceration, venous eczema).**
Class 4b:	**Skin changes ascribed to venous disease (e.g. pigmentation with palpable induration and no previous ulceration).**
Class 5:	Skin changes as in classes 4a or 4b with healed ulceration.
Class 6:	Skin changes as in classes 4a or 4b with active ulceration.

Table 2.10.1. Modified clinical classification of lower limb chronic venous disease.

SECTION III

Studies

3.1 STUDY I

Standardising of the calibration and measurement techniques of a newly developed tissue tonometer for use in analysing the mechanical properties of skin changes in patients with lower limb chronic venous disease.

3.1.1 Rationale

The clinical sequelae of CVD of the lower limbs range from oedema, haemosiderosis and pigmentation, to lipodermatosclerosis (LDS) and venous ulceration. The severity of the induration or skin hardness is difficult to assess objectively. A number of studies have used measurements of the area of lipodermatosclerosis, but judging the edge of the lesion is often very difficult and leads to large variance in the data. Clinical assessment of lipodermatosclerosis is the most frequently used method, applying the clinical skin severity score index adapted from studies of patients with systemic sclerosis (scleroderma) by Kahaleh et al in 1986. This is very subjective and unsuitable for use as an outcome measure in clinical studies. A durometer has also been used to determine the degree of induration in LDS by Romanelli and Falanga (Romanelli et al, 1995). Previous attempts to objectively measure the degree of skin hardness in other conditions, particularly systemic sclerosis (scleroderma), have included skin biopsies (Rodnan et al, 1979), skin elastometers (Bjerring et al, 1985; Ballou et al 1990), ultrasonography (Myers et al, 1986), magnetic resonance imaging (Richards et al, 1991) and $tcPo_2$ measurements (Silverstein et al, 1988). The disadvantages of the above methods are that they require expensive equipment or considerable experience and skin biopsies are invasive and are not practical for sequential measurements. At present, there is no standardised objective method of assessing the degree of skin changes seen in patients with lower limb chronic venous disease. A tissue tonometer has been developed in our laboratory for the objective assessment and quantification of the skin changes seen in these patients. A computerised tissue tonometer (see section 1.5.2 for details) was developed at our laboratory (UCL Hospitals, Middlesex Hospital Vascular laboratory, 1995) to objectively assess the mechanical properties of the skin changes in the gaiter area of patients with lower limb chronic venous disease.

3.1.2 <u>Aims</u>

To standardise the calibration, repeatability and reproducibility of the tonometry measurements.

To investigate whether the tonometer can differentiate between the mechanical properties of the skin in normal controls and in patients with lower limb chronic venous disease.

3.1.3 Methodology

3.1.3.1 Calibration check

The tonometer's calibration was checked using 4 brass discs of different thickness as measured by micrometer before and after 6 hours of recording on a 25% glycerol soaked sponge tissue (natural sponge cell, Boots plc) as described in section 2.8.

3.1.3.2 Calibration reproducibility

A calibration reproducibility check was done before and after 5 minutes of recording on compression sponges soaked in 25% and 50% of glycerol to ensure that there was no significant drift in signal output between measurements. Thirty recordings were made with fifteen sponges soaked in 25% glycerol and the other fifteen sponges soaked in 50% glycerol.

3.1.3.3 Subject preparation

All subjects were examined in the supine position with the test limb externally rotated and the knee slightly flexed so that the tissue tonometer could rest on the gaiter area as described in section 2.8. Three different sites were measured in the gaiter area to obtain a mean value as described in section 2.8 at 6cm, 10cm and 12cm above the medial malleolus. If an open ulcer is present at the above sites, the nearest area of skin with no open wound is used and the location noted for measurements on sequential visits. Identical sites were measured on the contralateral limb using the specially calibrated stand with a fixed marker pen (see section 2.5). This procedure was repeated on the other limb using the same heights noted on the calibrated column and the same landmarks to ensure identical sites are marked. With three identical sites marked in both lower limbs, the subjects were then examined by tissue tonometry as described in section 2.8.

3.1.3.4 Selection of attached weight and plunger diameter

Measurements were carried out on the gaiter area of the lower limb of a normal control subject (CEAP classification, C_0) using different diameters of plungers and different

weights attached as described in section 2.8 to see the differences between the pre-selected weight and plunger diameter and other weights and diameters. The displacement versus time traces obtained were analysed using the computer software to calculate the distance and rate constant parameters in all three phases.

3.1.3.5 Reproducibility and repeatability studies in normal controls and patient groups

Reproducibility studies using the tissue tonometer were carried out on normal controls and in patients with lower limb chronic venous disease to assess the reproducibility of the parameters measured.

Repeatability studies on one normal control was also performed.

Five normal controls (2M:3F; mean age of 31 years; range: 24-36 years) with no evidence of lower limb venous disease and three patients with lower limb chronic venous disease (1M:2F; mean age of 65 years; range 60-71 years) were investigated by tissue tonometry. Measurements of skin compliance were made on three consecutive days at the same time of day and on the same site, marked with a permanent black marker pen. The corresponding sites were measured on all the subjects and the site location was reproduced using the specially designed calibrated column stand and an indelible marker pen.

Same day repeatability studies were done on one normal control subject (female; age 29 years) with the same tissue tonometry site used and measured four times at 120 minute intervals with the subject instructed to carry on with her normal daily activities between measurements.

3.1.3.6. To differentiate between normal controls and patient groups with significant lower limb chronic venous disease by tissue tonometry.

Eighty six limbs of 69 patients (35F:34M; mean age of 62 years; range: 28-90 years) and 42 limbs of 37 normal controls (14F:23M; mean age of 42 years; range: 20-90 years) attending the vascular laboratory for full non-invasive venous assessments were investigated and the presence or absence of venous disease was confirmed by duplex ultrasonography. Resting ankle brachial pressure indices (ABPI) were also measured to eliminate any arterial component.

Differences in the tissue tonometry parameters between the young and old normal control groups (n = 42 limbs) were also investigated - twenty one limbs of the C_0 group with a mean age of 28 years and 21 limbs with a mean age of 55 years. Patients were

assigned to one of three clinical groups by the author based on the CEAP method of classification: normal controls were assigned C_0- no visible or palpable signs of venous disease, C_3-oedema, C_4-pigmentation and lipodermatosclerosis. The C_4 group was sub-divided into C_{4a} - pigmentation alone without palpable induration and C_{4b} - skin changes with palpable induration (see section 2.10 for details).

The patients in this study were deliberately selected with extreme clinical symptoms so as to check the ability of the tissue tonometer to differentiate between normal controls and patients with clinically significant oedema and skin changes.

The tissue tonometer was positioned on the gaiter area of the lower extremity with the subjects examined in the supine position as described earlier. Three different sites were measured in each subject to obtain a mean value. Identical sites were measured on the contralateral limb and in the normal control limbs using the specially calibrated stand as described earlier.

3.1.3.7. Data Analysis

Non-parametric analysis of the confidence intervals for the difference between the medians between normal controls and patient groups for the sample population were undertaken using the method described by Campbell and Gardner (Campbell et al, 1989).

The overall coefficient of variation (COV %) was analysed for normal controls and patient groups in the reproducibility study.

The overall coefficient of variation (COV %) was analysed for the repeat measurements on the same day performed on the same subject.

The distance and rate constant parameters were plotted as scattergrams for all the different classification groups and medians and interquartile ranges were used as descriptors. The Mann Whitney U test was applied to test for statistical significance.

3.1.4. Results

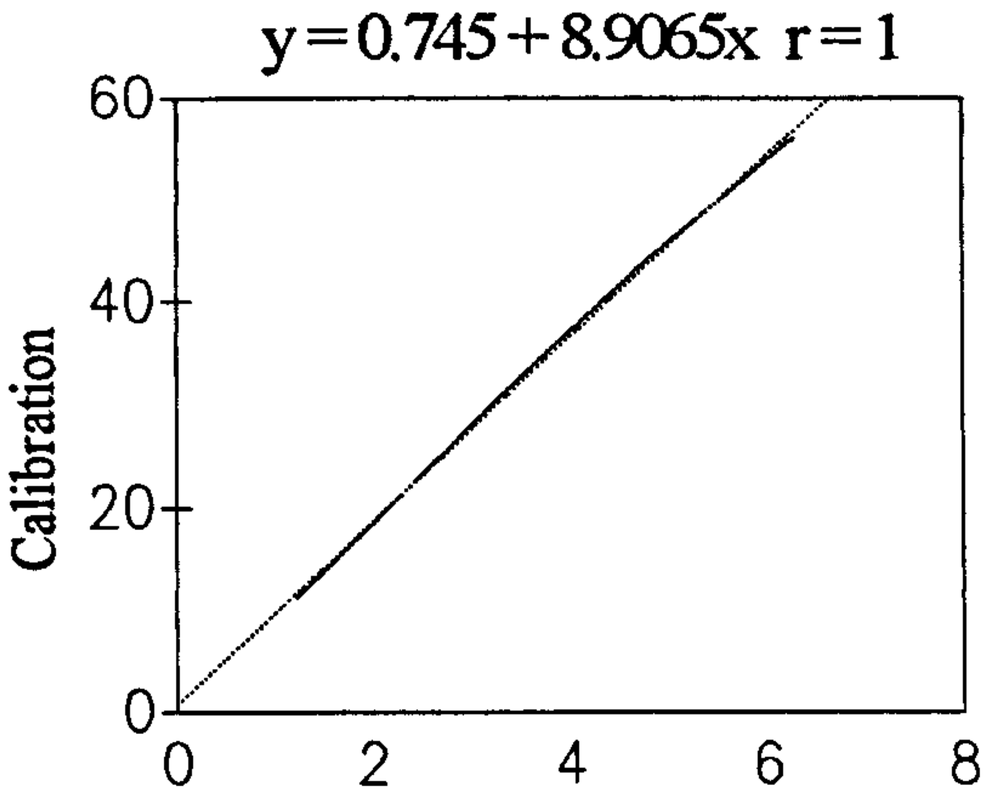


Figure 3.1.1. Calibration curve for tissue tonometer (screen deflection as a function of plunger displacement) before recording on the sponge tissue.

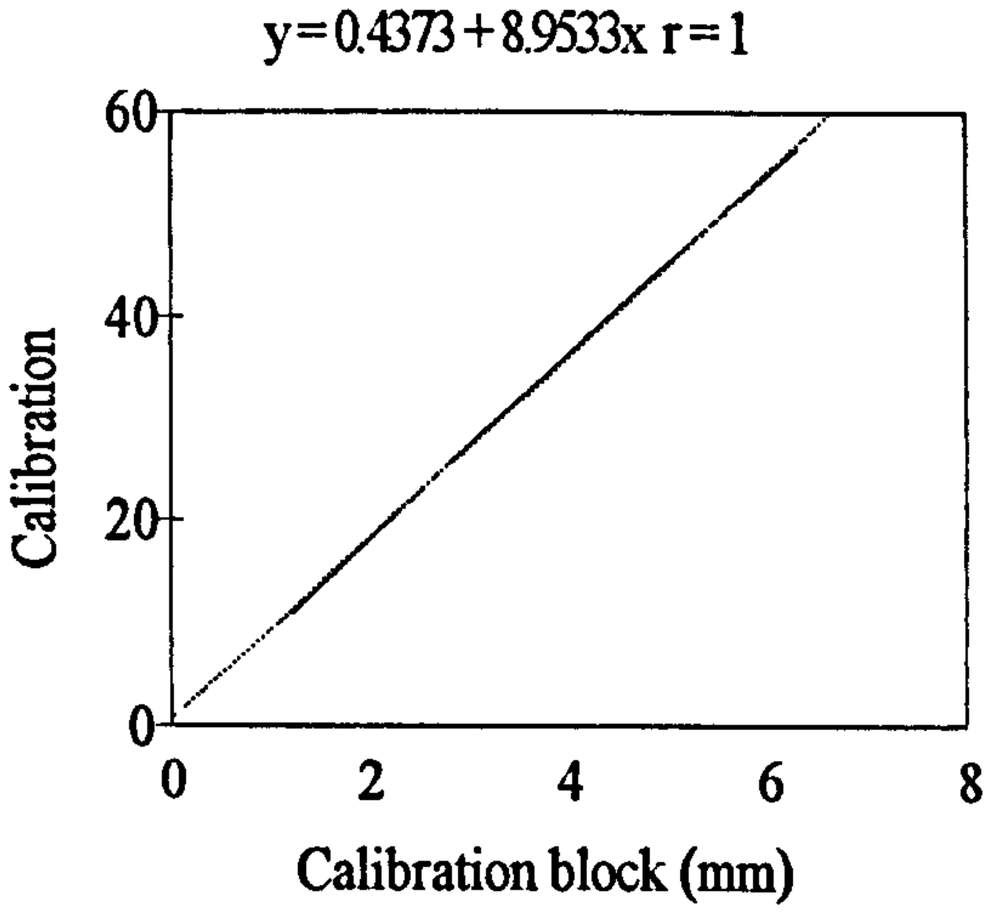


Figure 3.1.2. Calibration curve for tissue tonometer (screen deflection as a function of plunger displacement) after recording on the sponge for 6 hours.

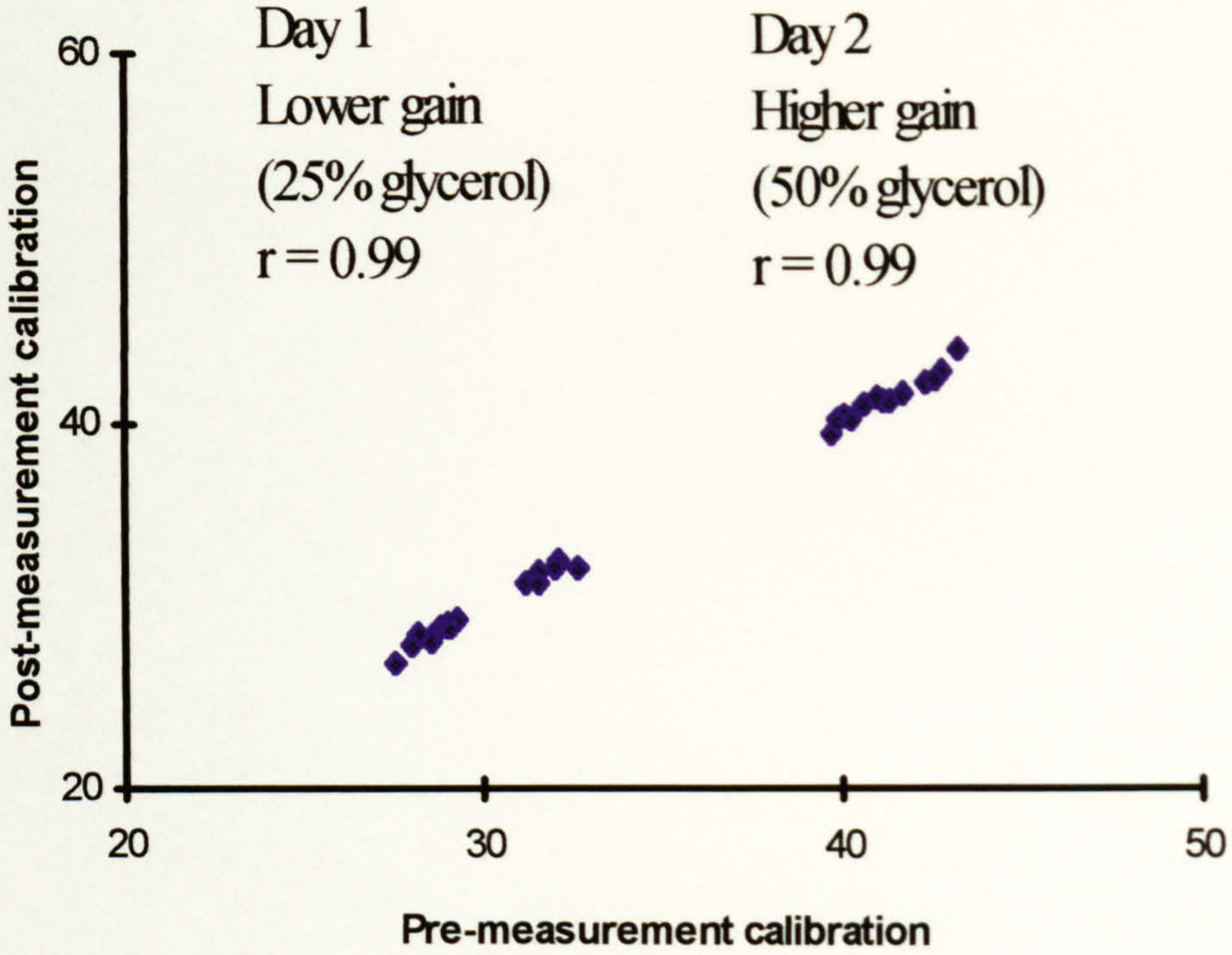


Figure 3.1.3 Pre- and post-measurement tonometry calibration readings after 5 minutes of recording on glycerol-soaked sponge.

	Phase I (0 - 1 sec)	Phase II (1 - 10 secs)	Phase III (10 - 300 secs)	Phase III (10 - 300 secs)
	Distance parameter X_0 (mm)	Distance parameter X_I-X_0 (mm)	Distance parameter X_I-X_0 (mm)	Rate constant parameter -1/Tau (seconds^{-1})
CEAP Hawaii classification	**median (IQR)**	**median (IQR)**	**median (IQR)**	**median (IQR)**
Control (C_0) (n = 42)	2.85 (2.39 - 3.22) ♣*	0.28 (0.19 - 0.37) ♦♣⊕	0.10 (0.02 - 0.18) ♣♦♦	114.65 (67.25 - 335.22) ♥
Oedema (C_3) (n = 28)	2.49 (1.58 - 3.13) *⊕	0.57 (0.44 - 0.80) ♣	0.56 (0.17 - 0.81) ♣	65.3 (51.15 - 94.90)
Pigmentation (C_{4a}) (n = 14)	2.35 (2.04 - 2.91) ♦	0.73 (0.48 - 0.90) ⊕	0.51 (0.28 - 0.65) ♦	56.1 (46.67 - 57.72)
LDS (C_{4b}) (n = 44)	0.81 (0.67 - 1.18) ♣♦⊕	0.42 (0.25 - 0.75) ♦	0.26 (0.07 - 0.47) ♣	41.8 (25.0 - 64.65) ♥
⊕♣♦♣♥ p<0.0005 (Mann Whitney u test)				
*p = 0.70 (Mann Whitney u test)				

Table 3.1.1 Tissue tonometry parameters - analysis for significance tests between normal controls and patient groups.

	Phase I (0 - 1 sec)	Phase II (1 - 10 secs)	Phase III (10 - 300 secs)	Phase III (10 - 300 secs)
	Distance parameter X_0 (mm)	Distance parameter X_I-X_0 (mm)	Distance parameter X_I-X_0 (mm)	Rate constant parameter -1/Tau (seconds^{-1})
CEAP Hawaii classification	**median (IQR)**	**median (IQR)**	**median (IQR)**	**median (IQR)**
Control (C_0) (young) (n = 21) mean = 28 yrs	2.71 (2.41 - 3.10) *	0.29 (0.20 - 0.36) *	0.12 (0.06 - 0.19) *	99.2 (68.6 - 290.2) *
Control (C_0) (old) (n = 21) mean = 55 yrs	2.94 (2.38 - 3.33) *	0.27 (0.18 - 0.38) *	0.05 (0.01 - 0.11) *	119.5 (58.0 - 379.3) *
*p = 0.17				

Table 3.1.2 Tissue tonometry parameters - analysis for significance tests between young and old normal controls.

	Phase I (0 - 1 sec)	Phase II (1 - 10 secs)	Phase III (10 - 300 secs)	Phase III (10 - 300 secs)
	Distance parameter X_0 (mm)	**Distance parameter X_I-X_0 (mm)**	**Distance parameter X_I-X_0 (mm)**	**Rate constant parameter -1/Tau (seconds^{-1})**
CEAP Hawaii classification	**median (CI)**	**median (CI)**	**median (CI)**	**median (CI)**
C_0 -C_{4b}	1.92(1.68 to 2.15)	0.20(0.09 to 0.30)	0.18(0.06 to 0.27)	68.6(41 to 115)
C_0 - C_{4a}	0.29(0.17 to 0.73)	0.42(0.26 to 0.58)	0.32(0.19 to 0.50)	63(28 to 204)
C_0 - C_3	0.40(0.06 to 0.86)	0.28(0.17 to 0.39)	0.43(0.19 to 0.65)	47.5(16 to 104)
C_{4a} - C_{4b}	1.58(1.26 to 1.95)	0.23(0.04 to 0.39)	0.16(0 to 0.30)	5(-20 to 10)
C_0(young) - C_0(old)	0.16(-0.59 to 0.36)	0.02(-0.07 to 0.10)	0.07(0.01 to 0.12)	0(-102 to 45.2)

Table 3.1.3 95% Confidence intervals (CI) for difference between medians in normal controls and patient groups.

	Phase I	Phase II	Phase II	Phase III	Phase III
Diameter of plunger and weight attached	**Distance parameter X_0 (mm)**	**Distance parameter X_I-X_0 (mm)**	**Rate constant parameter -1/Tau (seconds^{-1})**	**Distance parameter X_I-X_0 (mm)**	**Rate constant parameter -1/Tau (seconds^{-1})**
4mm,10g	1.29	0.15	3.13	0.22	74.9
4mm,30g	**3.07**	**0.20**	**2.92**	**0.25**	**60.4**
4mm,60g	3.83	0.35	2.79	0.49	65.3
4mm,90g	3.63	0.06	2.05	0.01	541.5
10mm,10g	0.89	0.01	4.66	0.04	146
10mm,30g	1.09	0.04	2.67	0.02	134.4
10mm,60g	2.21	0.49	1.57	0.12	83.5
10mm,90g	2.61	0.15	3.01	0.12	79.1

Table 3.1.4 Distance and rate constant parameters obtained with different weights and plungers - sponge in glycerol.

	Phase I (0 - 1 sec)	Phase II (1 - 10 secs)	Phase III (10 - 300 secs)	Phase III (10 - 300 secs)
	Distance parameter X_0 (mm)	Distance parameter X_I-X_0 (mm)	Distance parameter X_I-X_0 (mm)	Rate constant parameter -1/Tau (seconds^{-1})
	COV (%)	COV (%)	COV (%)	COV (%)
Patient group (n=3)	3.8	18.5	86.5	56.9
Normal control group (n=5)	8.0	61.5	108.2	144.3

Table 3.1.5 Reproducibility study in patient group and normal control group for the various parameters.

	Phase I (0 - 1 sec)	Phase II (1 - 10 secs)	Phase III (10 - 300 secs)	Phase III (10 - 300 secs)
	Distance parameter X_0 (mm)	Distance parameter X_I-X_0 (mm)	Distance parameter X_I-X_0 (mm)	Rate constant parameter -1/Tau (seconds^{-1})
	COV (%)	COV (%)	COV (%)	COV (%)
Normal control (n=1)	4.5	22.1	30.8	10.8

Table 3.1.6 Same day repeatability study in one normal control allowing 2 hours between measurements (x4).

Distance parameter - phase I (0 - 1 sec) during initial compliance phase

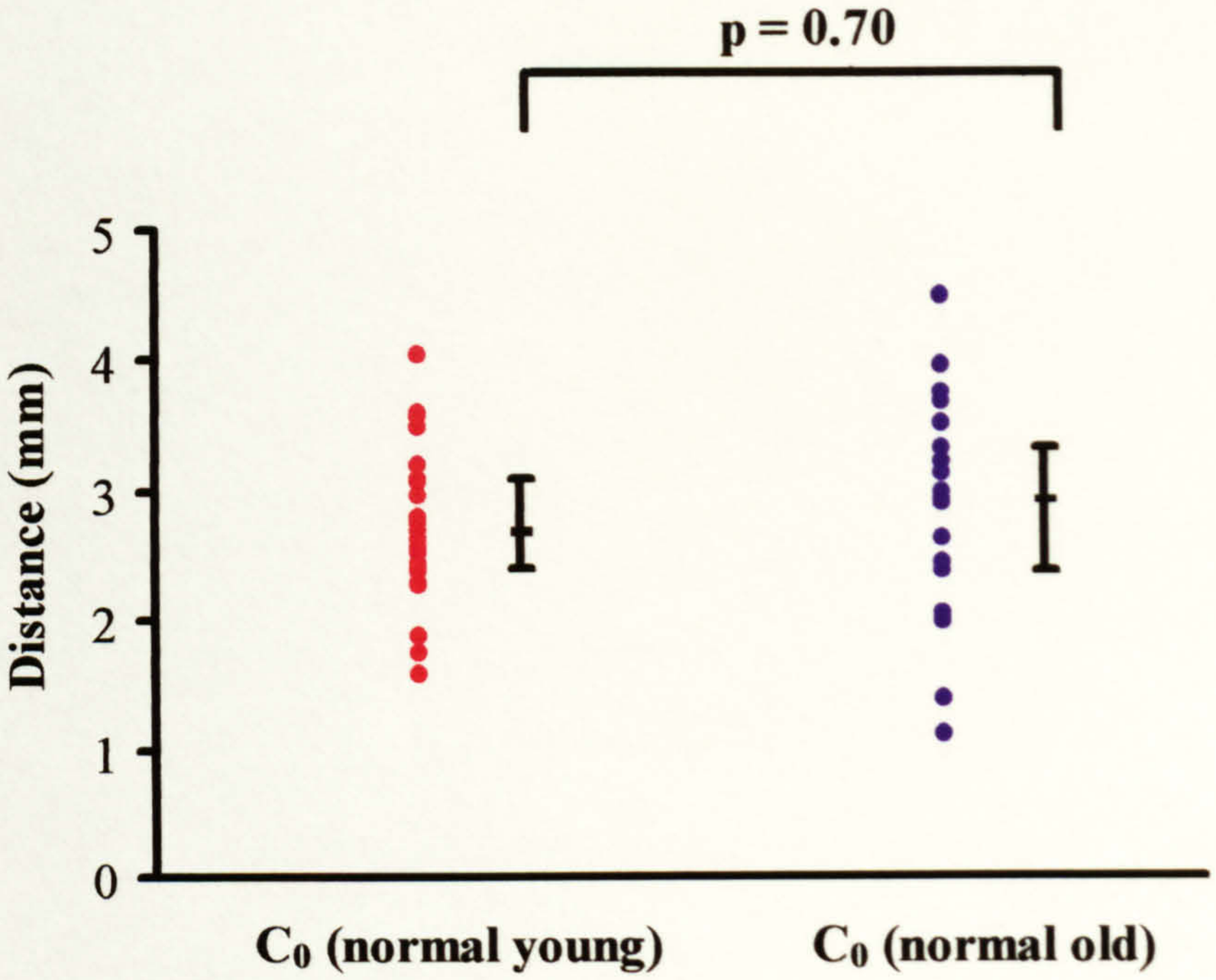


Figure 3.1.4 Distance travelled by the plunger in phase I, X_0, the initial rapid indentation phase, which occurs within 1 second and reflects the skin compliance. There is no statistically significant difference between the young and old normal controls.

Distance parameter - phase II (1 - 10 secs)

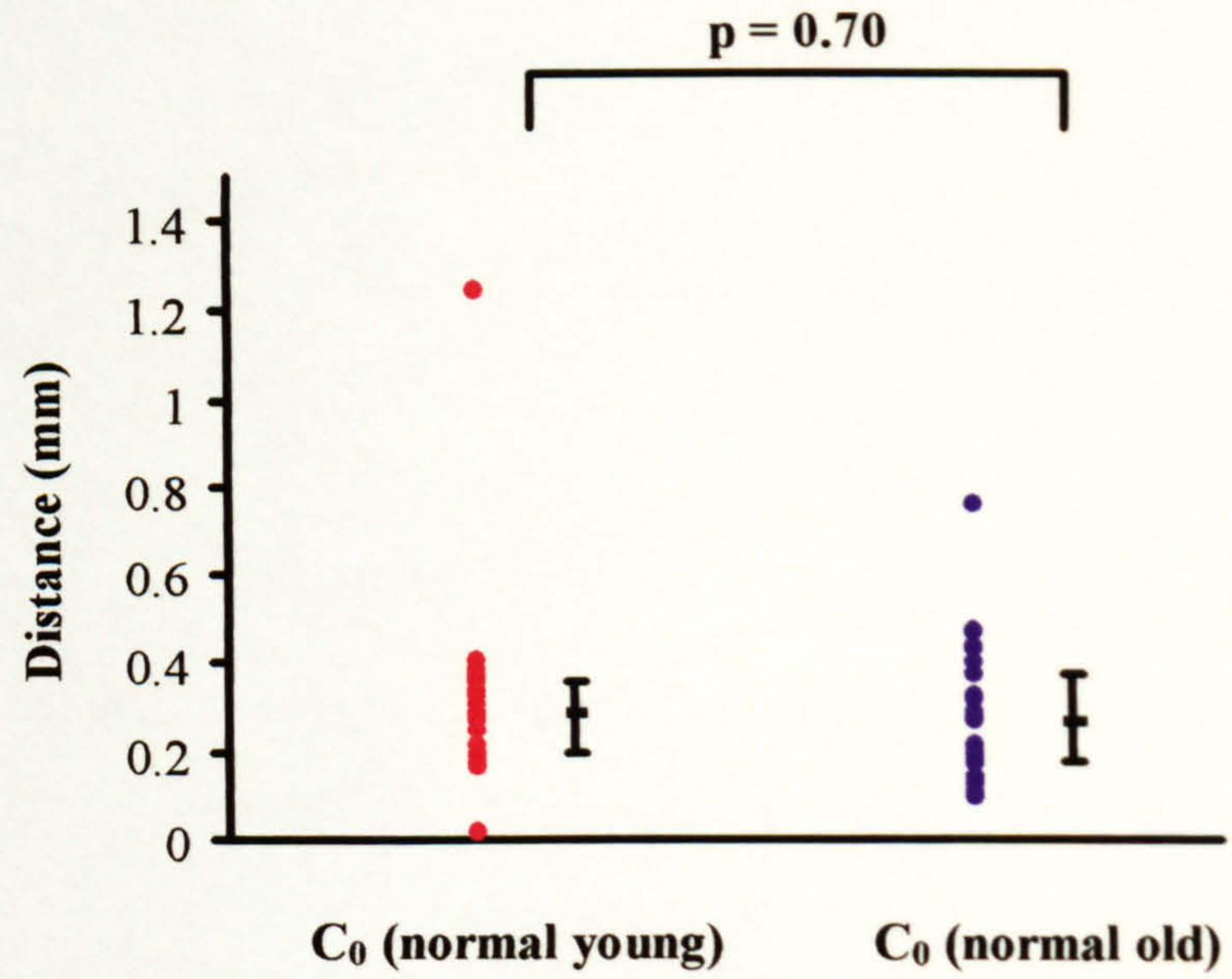


Figure 3.1.5 Distance travelled by the plunger in phase II, the subsequent slower indentation phase, X_I-X_0, which occurs between 1 and 10 seconds and probably reflects tissue oedema. There is no statistically significant difference between the young and old normal controls.

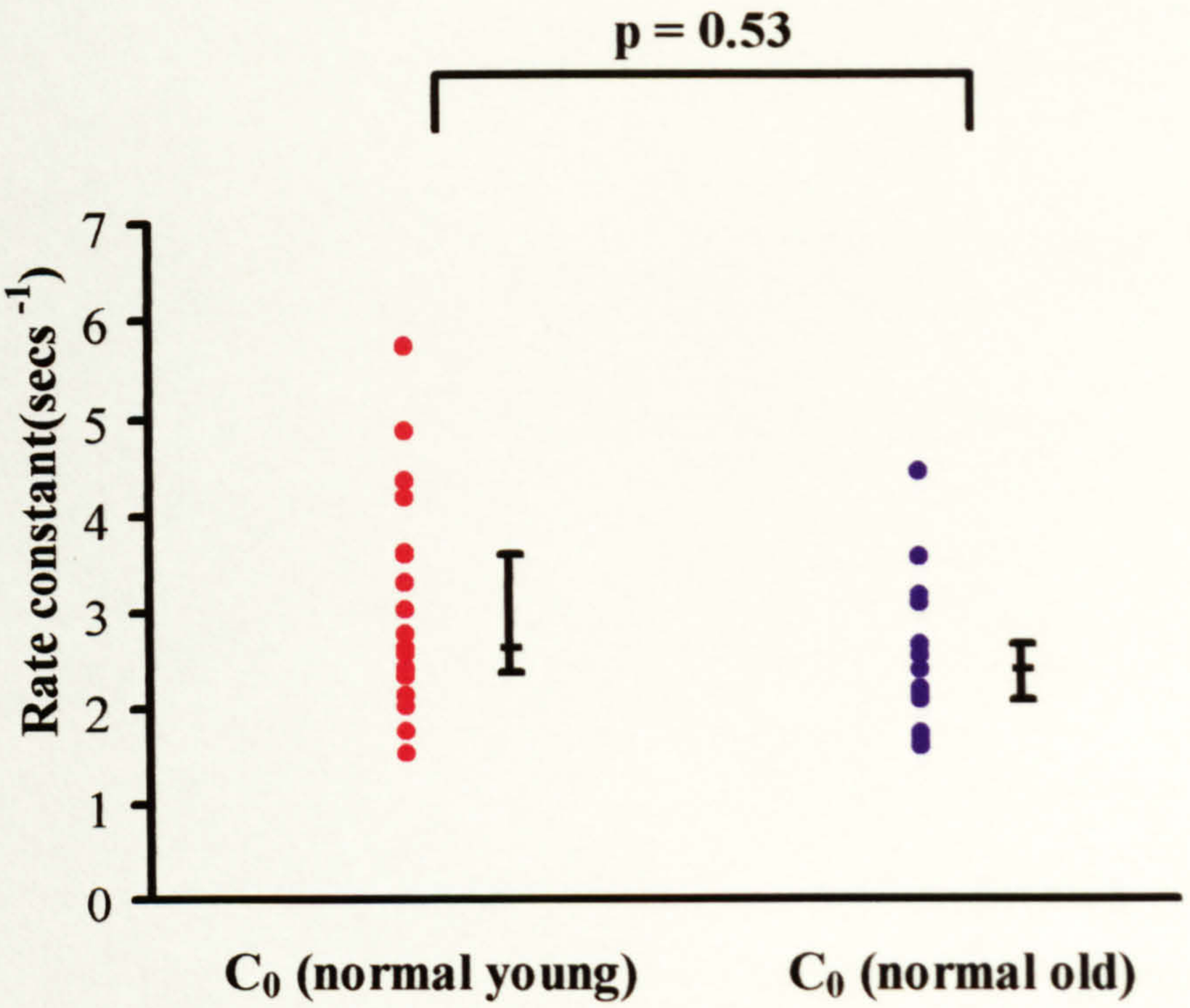


Figure 3.1.6 Rate constant parameter , -1/Tau, during phase II, the subsequent slower indentation phase, which occurs between 1 and 10 seconds and probably reflects tissue oedema. There is no statistically significant difference between the young and old normal controls.

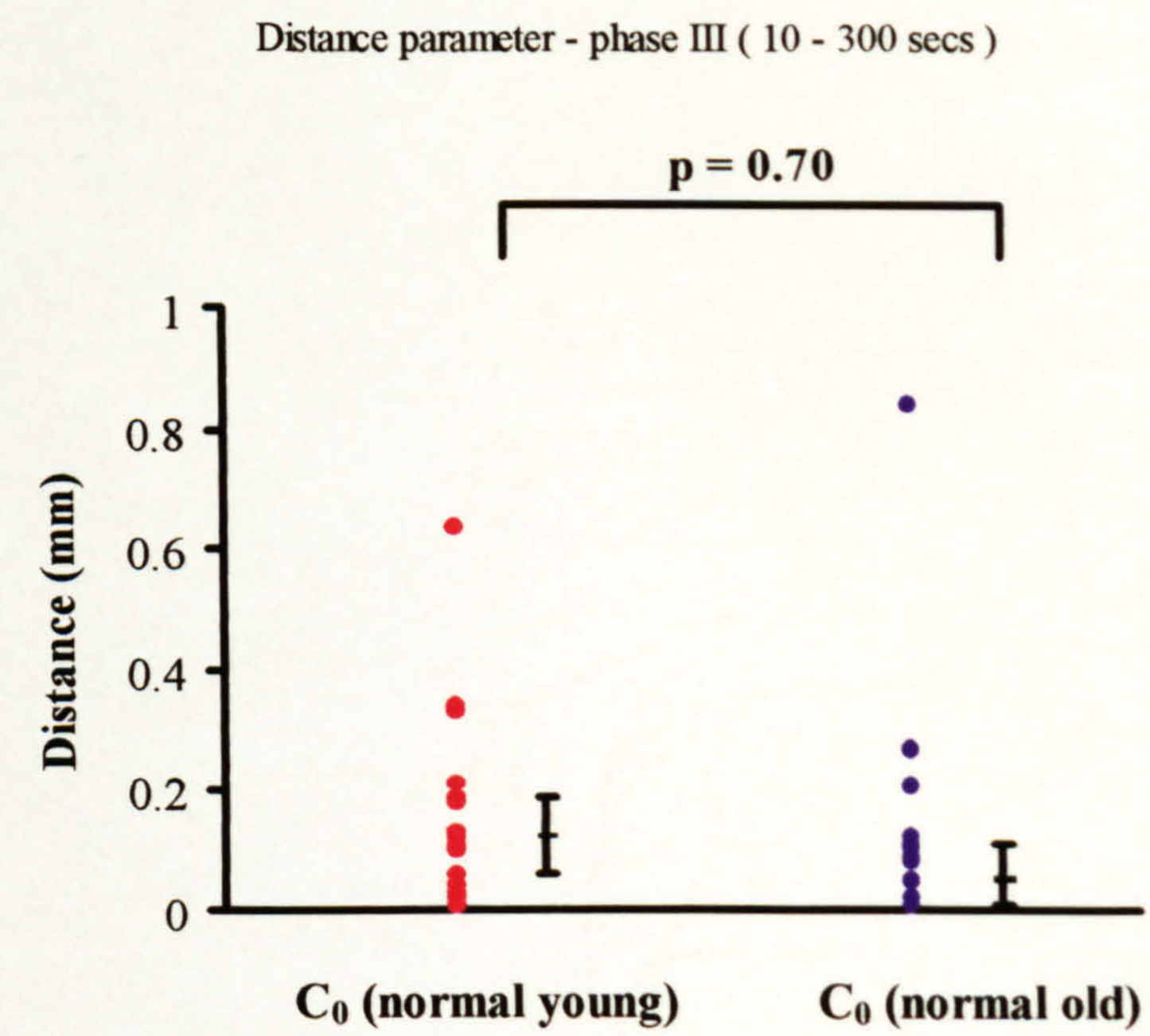


Figure 3.1.7 Distance travelled by the plunger in phase III, the subsequent slower indentation phase, which occurs between 10 and 300 seconds and probably reflects tissue oedema. There is no statistically significant difference between the young and old normal controls.

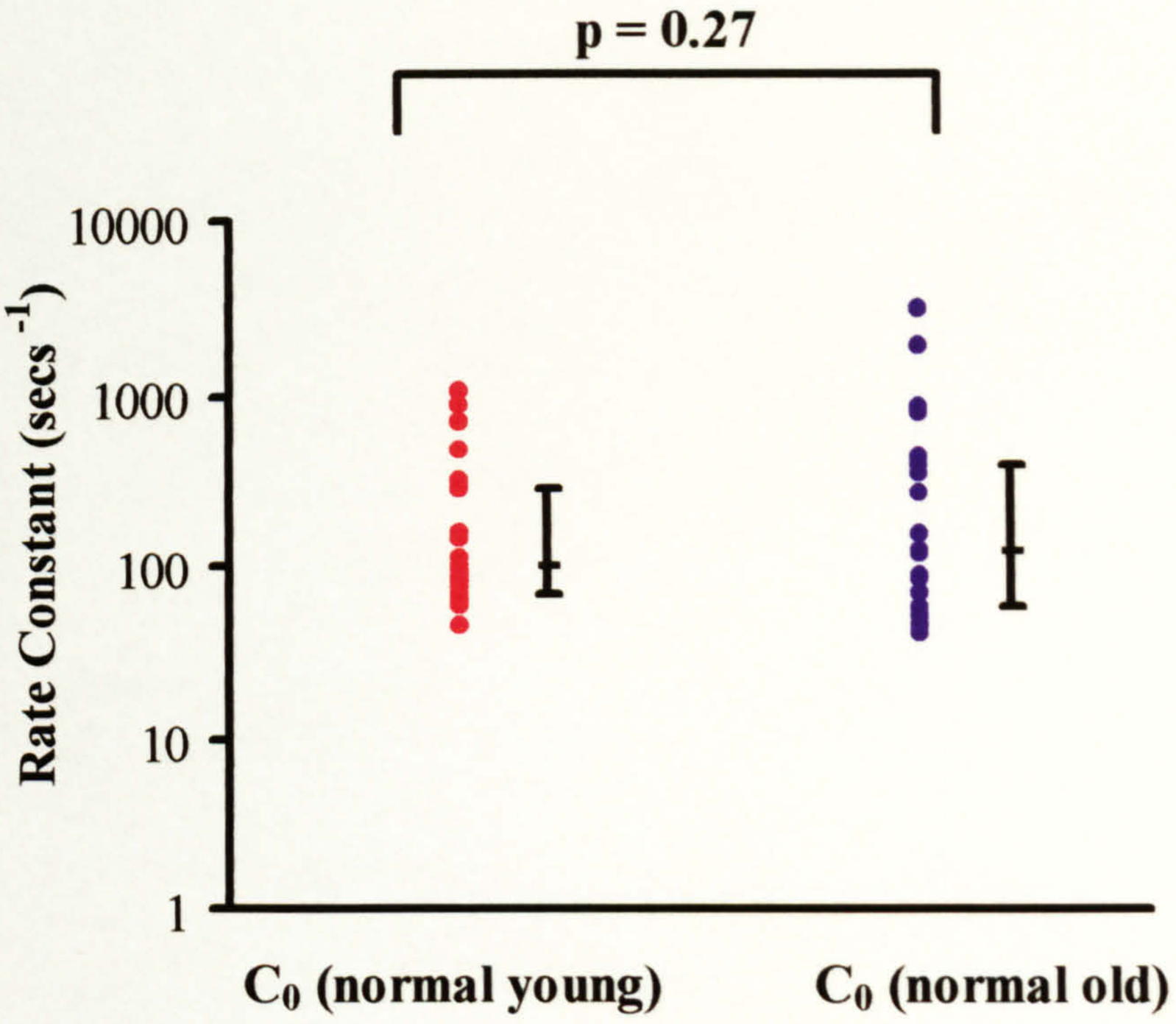


Figure 3.1.8 Rate constant parameter, -1/Tau, during phase III, the subsequent slower indentation phase, which occurs between 10 and 300 seconds and probably reflects tissue oedema. There is no statistically significant difference between the young and old normal controls.

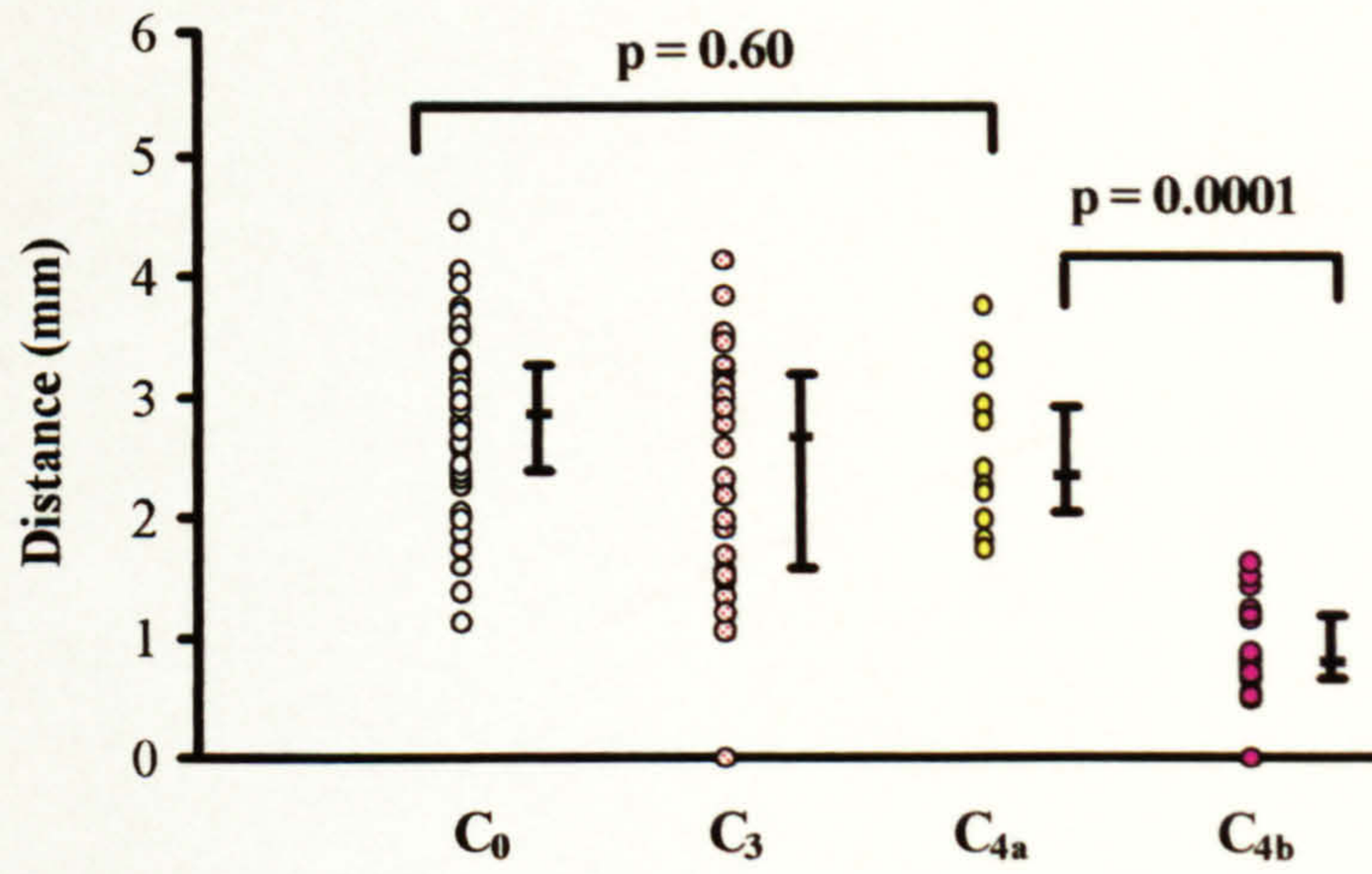


Figure 3.1.9 The skin compliance is significantly reduced in the LDS group as compared to the normal controls, oedema and pigmentation alone groups. There is no statistical significant difference in skin compliance between the normal control, pigmentation alone and oedema groups.

Distance parameter - phase II (1 - 10 secs)

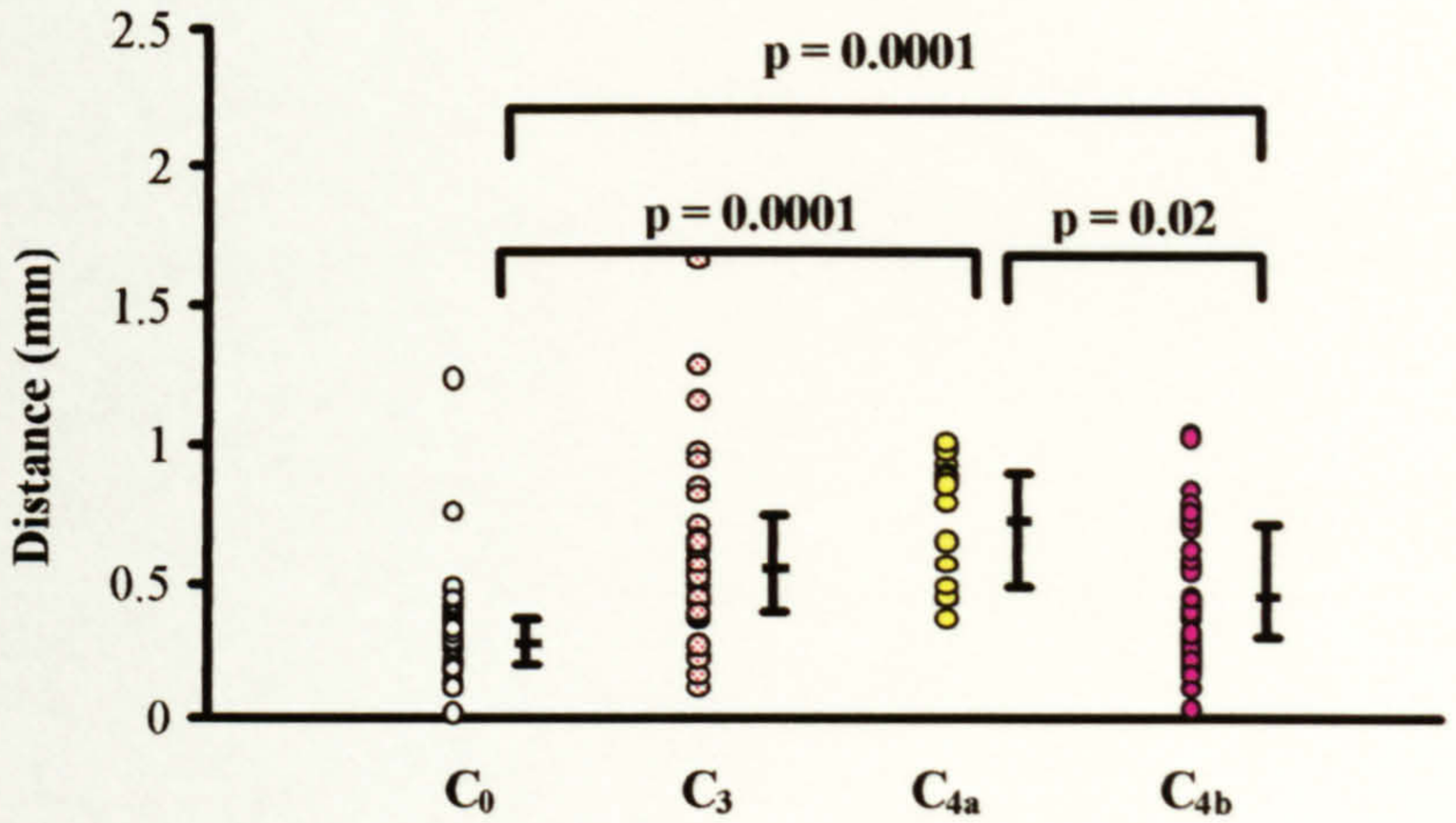


Figure 3.1.10 The plunger travels much further in the oedema and pigmentation alone groups as compared to the normal control group and this reaches statistical significance. This phase probably reflects tissue oedema.

Rate constant parameter - phase II (1 - 10 secs)

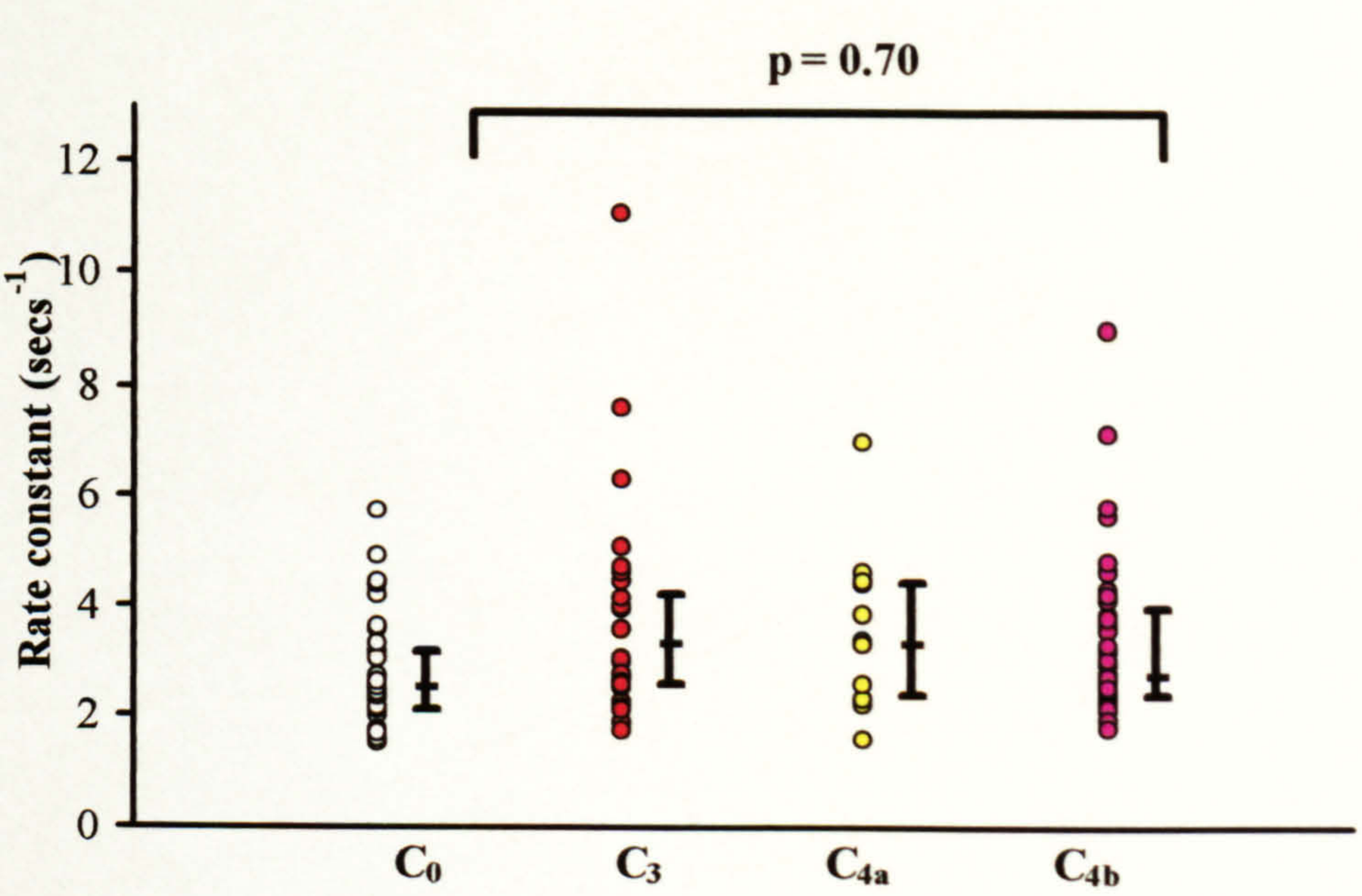


Figure 3.1.11 There is no statistically significant difference in the rate constant parameter between the groups in phase II.

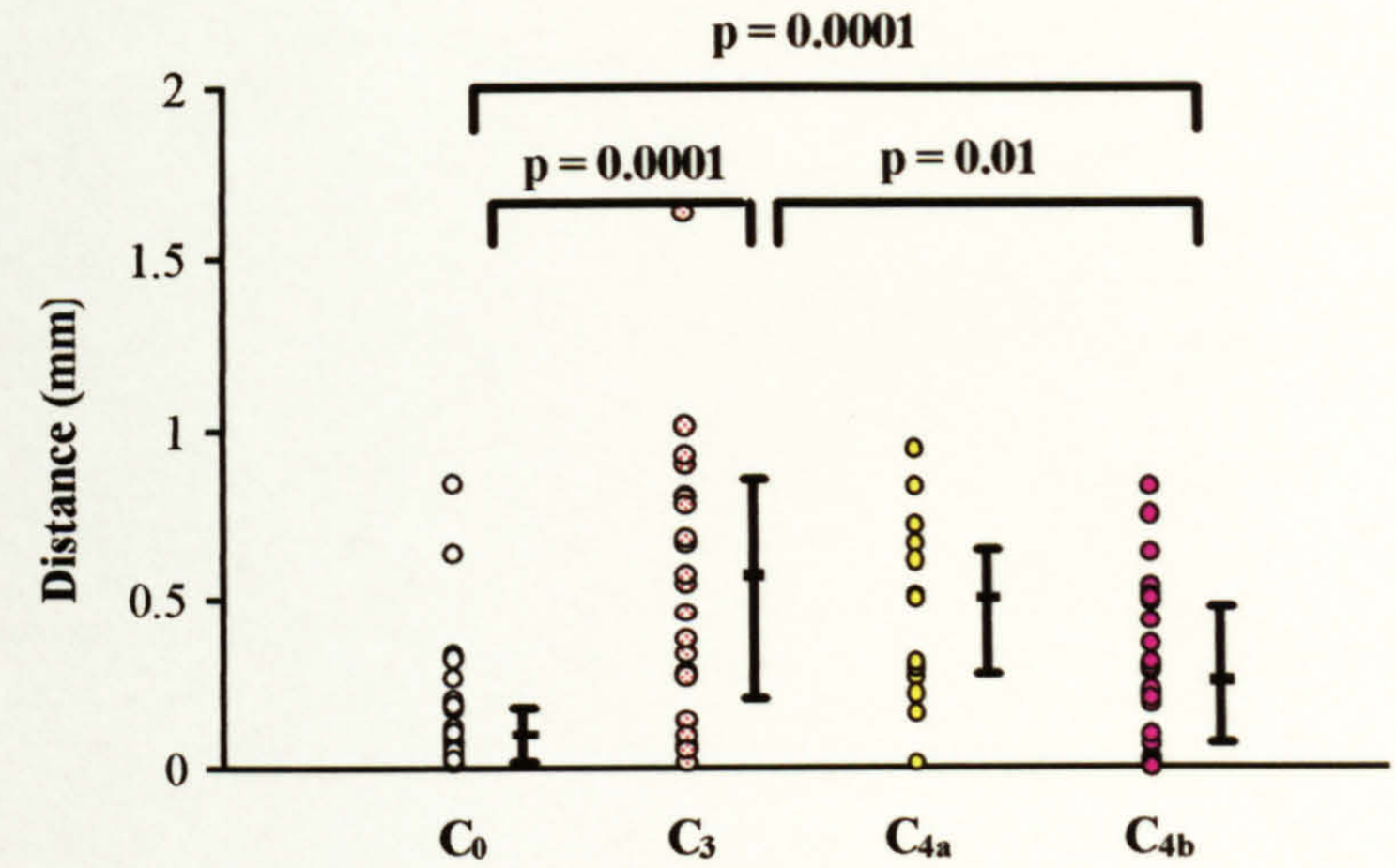


Figure 3.1.12 The plunger travels much further in the oedema, LDS and pigmentation alone groups as compared to the normal control group and this reaches statistical significance. This phase probably reflects tissue oedema. There is no significant difference between the LDS, oedema and pigmentation alone groups probably reflecting the presence of tissue oedema.

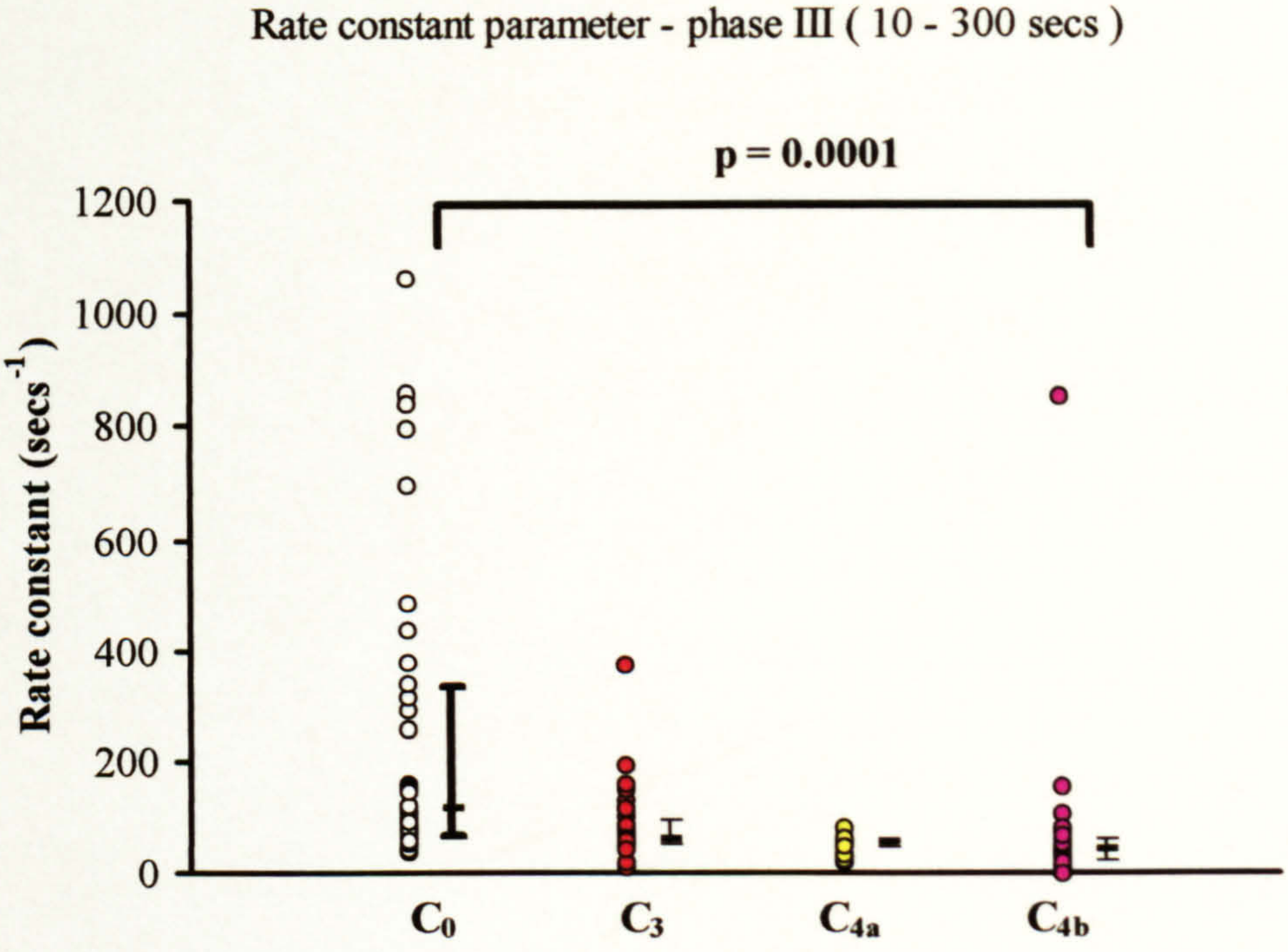


Figure 3.1.13 There is a significant difference in the rate constant parameter between the normal control group and the other three groups as the plunger stops sinking into the tissues much earlier in the normal control group as compared to the other three groups.

A 4mm diameter plunger and a 30g weight were used in all the tonometry measurements in this thesis.

In the calibration check study, there was perfect correlation (r=1.00) between the screen deflection as a function of the plunger displacement using different thickness of metal blocks before and after 6 hours of continuous recording on a piece of sponge soaked in glycerol (figures 3.1.1 and 3.1.2).

In the calibration reproducibility check study, there was perfect correlation between the pre-calibration and post-calibration (r=0.99) figures for both measurements (figure 3.1.3).

There was good reproducibility in the measurement of the skin compliance, X_0 , in normal subjects (COV of 8%) and in patients (COV of 3.8%) on consecutive days (table 3.1.5). The distance and rate constant parameters in phases II and III showed much greater variation on different days.

There was good repeatability in the measurement of the skin compliance X_0 with a COV of 4.5% on the same normal control subject after four measurements were repeated on the same day (table 3.1.6) and again the other distance and rate constant parameters in phases II and III proved to be less reproducible than the skin compliance parameter.

There were no statistically significant differences in the skin compliance and in the distance and rate constant parameters in phases II and III between the normal young controls, mean age 28 years, and the normal old controls, mean age 55 years (see table 3.1.2 and figures 3.1.4 to 3.1.8).

There was a significant difference in the skin compliance measured during phase I between normal controls (C_0) and patients with liposclerotic skin changes and palpable induration (C_{4b}) (table 3.1.1) by as much as three-fold (figure 3.1.9). There was a substantial difference in the skin compliance between the C_{4a} group and the C_{4b} group (figure 3.1.9). There was no significant difference in the skin compliance between the normal controls C_0 and the C_3 (oedema) and C_{4a} groups.

During phase II, the distance travelled by the plunger was significantly further in the C_3, C_{4a} and C_{4b} groups than in the C_0 group (figure 3.1.10). There was no significant difference in this parameter between the C_3, C_{4a} and C_{4b} groups. During phase II, there was no significant difference in the rate constant parameter between the C_0, C_3, C_{4a} and C_{4b} groups (figure 3.1.11).

During phase III, the distance travelled by the plunger was significantly further in the C_3, C_{4a} and C_{4b} groups than in the C_0 group (figure 3.1.12). Interestingly, in the C_{4b}

group, the plunger travels less than in the C_3 and C_{4a} groups though clinically there was significant oedema present.

During phase III, the rate constant parameter was significantly higher in the C_0 group as compared to the C_3, C_{4a} and C_{4b} groups (figure 3.1.13). There were no significant differences in the rate constant parameter between the C_3, C_{4a} and C_{4b} groups.

3.1.5 Discussion

Previously assessment of skin damage in venous disease has relied on clinical means alone, although Falanga has reported the use of a durometer to add objectivity to this measurement. This study set out to evaluate a more rigorous method of investigating the mechanical properties of the skin and its response to mechanical deformation. A weighted plunger was equipped with a measuring and recording system and the traces recorded from patients and control subjects were assessed.

The measuring system itself proved to be very reliable and introduced no extraneous errors. In the calibration check study, there was perfect correlation (r=1.00) between the screen deflection as a function of the plunger displacement using different thickness of metal blocks before and after 6 hours of continuous recording on a piece of sponge soaked in glycerol (figures 3.1.1 and 3.1.2). This demonstrated that the calibration of the tissue tonometer was very reproducible and did not drift with time. In the calibration reproducibility study, there was perfect correlation between the pre-calibration and post-calibration (r=0.99) figures for both measurements (figure 3.1.3).

In the clinical studies great care was taken to ensure that the same region of skin was evaluated on each occasion. There was good reproducibility in the measurement of the skin compliance, X_0 , in normal subjects (COV of 8%) and in patients (COV of 3.8%) on consecutive days (table 3.1.5) which was not affected by the physiological variables (oedema, blood, subcutaneous layer thickness) and proved to be a stable parameter. The distance and rate constant parameters in phases II and III showed much greater variation and are clearly much less reliable in assessing the severity of skin damage. There was good repeatability in the measurement of the skin compliance X_0 with a COV of 4.5% on the same normal control subject after four measurements were repeated on the same day (table 3.1.6) and again the other distance and rate constant parameters in phases II and III proved to be less reproducible than the skin compliance parameter. These findings suggest that the plunger displacement in the first second is the most satisfactory

measure to use, however, the results from all parameters are included in this and subsequent chapters for completeness.

The aim in this investigation was to establish whether an objective measurement of the mechanical properties of the skin could improve on clinical assessment. I therefore investigated patients venous disease, comparing those with skin changes to those without. Most classifications of the clinical severity of venous disease include all types of skin changes (eczema, haemosiderosis and lipodermatosclerosis) in one group. This is clearly an oversimplification since patients with eczema are at low risk of leg ulceration and those with lipodermatosclerosis are at high risk. It became apparent in the preliminary work that measurements differed greatly in the patients with palpable induration from those with only skin pigmentation. The CEAP clinical classification assigns all patients with skin changes to group C_4. I subdivided this group for the purpose of these studies into C_{4a} for patients with pigmentation alone and C_{4b} for patients with skin changes and palpable induration of the skin and subcutaneous tissues. This revealed large differences between the C_{4a} and C_{4b} groups for the phase I measurement. This clearly showed considerable reduction in skin deformability, reflecting the clinical sensation of induration when palpating the skin. The difference lay between the C_{4b} patients and all other groups, and the phase I measurement was identical between young and old patients, confirming that any changes in skin elasticity with advancing age did not alter this measurement.

The phase II measurements (1 – 10 seconds) showed a difference between the controls and all venous disease groups for the distance parameter, although there was substantial overlap between groups. Assuming that the mechanical elasticity has been taken up within the first second after application of compression, this probably reflects the displacement of fluid from the tissues. The duration of this phase is fairly short so it is unlikely that is represents substantial movement of extracellular fluid and I believe that it probably reflects movement of blood in veins (large and small) in which the applied force was sufficient to displace the venous blood. The distance moved by the plunger in this phase was greater in all patient groups than control subjects, consistent with the increased venous volume in patients with venous incompetence of any type. However, the repeatability of these measurements was not very good, reducing their value for the assessment of the severity of venous disease. During phase II, there was no significant difference in the rate constant parameter between the C_0, C_3, C_{4a} and C_{4b} groups (figure

3.1.11) suggesting that the type of fluid, probably blood, that is being translocated beneath the plunger is similar in all the groups.

The phase III measurements (10 – 300 seconds) represent a period where the venous blood has been displaced and the travel of the plunger must be attributable to displacement of extracellular fluid, including any oedema. Travel of the plunger was least in the control group and greater in the patients with venous disease, without lipodermatosclerosis. This is consistent with these patients having an increased amount of tissue fluid due to their venous disease. Interestingly, the travel during phase III in the C_{4b} patients was less than that for the less severely damaged skin. This may reflect an interaction of this measurement with the loss of skin compliance. This is greatly reduced in patients with LDS and probably limits the plunger travel, even when substantial amounts of oedema are present. During phase III, the rate constant parameter was significantly higher in the C_0 group as compared to the C_3, C_{4a} and C_{4b} groups (figure 3.1.13). This was because in the C_0 group, the plunger ceased to sink into the tissues much earlier in the phase as there was less tissue fluid to displace. A reduced rate constant indicates that the final value was reached more slowly, that is indicating more tissue fluids present beneath the plunger in the C_3, C_{4a} and C_{4b} groups. There were no significant differences in the rate constant parameter between the C_3, C_{4a} and C_{4b} groups, suggesting that the type of fluid being translocated beneath the plunger in the patient groups is mainly interstitial fluid.

It has been previously demonstrated that the mobility of fluid flow in the subcutaneous tissue increases substantially with oedema (Guyton et al, 1971). They explained that the fluid flow through the tissue spaces is normally restricted by solid elements which are packed upon one another and by the high viscosity of the gel-like matrix embedded in a network of collagen fibres (Brace and Guyton, 1979). As oedema develops, the tissue spaces become greatly enlarged and fluid replaces the gel in the tissue spaces. The mobility of the interstitial fluid should indicate the degree of oedema. A non-invasive instrument was developed (Mridha et al, 1986), using a well defined step compression over an area of skin and continuously recording the resistive force of the tissue, to assess subcutaneous oedema and compared normal skin and oedematous skin. He showed that fluid flow out of the compressed oedematous tissues was quicker than from normal tissues and measurements from the contralateral site of subjects with unilateral oedema provided further evidence that the volume and flow rate of the translocated fluid was a function of oedema. Their results were comparable to the mathematical analysis of fluid

flow from a compressed tissue area (Guyton et al, 1971) and concluded that the lower resistance to fluid motion in oedematous tissue was due to the lower viscosity of the interstitial fluid, enlarged interstitial spaces and less compacted structure of the solid elements in oedematous tissues than in normal tissues. In oedematous tissues, the viscosity of the free fluid is lower, the amount of free fluid is greater and the spaces between the fibres is greater than in normal tissues making fluid flow easier. When oedema with a low surface tension is pitted, the depression follows the surface of the pressure head closely but for stiffer tissues (as in LDS skin), the skin will not follow as well and the calculation of the translocation volume will be less accurate. However, translocation of fluid does take place as shown by the force decay.

Confidence interval analysis confirms that the X_0 distance provides the most reliable parameter for distinguishing between the skin changes in patients with liposclerotic skin changes (C_{4b}) compared to those with haemosiderosis alone (C_{4a}). This parameter also has a low coefficient of variation. This measurement is likely to be a useful parameter and potential candidate as a surrogate endpoint in studies of the efficacy of treatments for chronic venous disease.

3.1.6 Conclusion

In the above study, I have shown that the tissue tonometer can objectively distinguish between the varying hardness of skin in patients with lower limb chronic venous disease. The patients were deliberately selected with advanced clinical signs of venous disease to fit into their respective clinical classification groups according to the CEAP clinical classification method. This was to assess if the tonometer could differentiate between the varying degrees of skin changes with increasing severity of the disease.

This study has shown that tissue tonometry measurement of the skin compliance provides an objective means of assessing skin changes in patients with chronic venous disease.

It has also shown that it was necessary to differentiate between patients in the group C_4,into patients with pigmentation alone and no palpable induration (C_{4a}) and patients with liposclerotic skin changes with palpable induration (C_{4b}) using the CEAP clinical classification method to get a more objective assessment of the skin changes that are present in patients with lower limb chronic venous disease. The tissue tonometry measurements of distance and rate constant parameters in the subsequent phases II and III may possibly reflect increased venous volume and extent of tissue oedema.

3.2 STUDY II

Use of a tissue tonometer *in vitro* to verify the application of a simple mathematical model in the analysis of the displacement versus time curves obtained from a sponge-in-glycerol model to explain its mechanical behaviour.

3.2.1 Rationale

The displacement versus time curves obtained from the tissue tonometer both *in vitro* (compression of glycerol soaked sponges (natural sponge, Boots plc)) and *in vivo* (normal control subjects and subjects with evidence of lower limb chronic venous disease), showed an initial rapid indentation phase followed by a subsequent slower indentation phase.

The model for an ideally visco-elastic material giving both an instant elastic response to loading and lacking plastic properties and which generates curves of the type described above, has been previously used to describe the effects of applying a loaded plunger on a sponge in glycerol model, which show visco-elastic properties, to explain its behaviour under compression (Bates et al, 1994). This simple mechanical model, the Kelvin-Hooke model, is a combination of a Kelvin element in series with an elastic element (Hooke element) (see section 1.5.3) which provides the two elastic spring constants (C_2 and C_1) and the viscous dashpot constant (k).

3.2.2 Aims

To standardise the tonometer *in vitro* using the sponge-in-glycerol model to obtain displacement versus time curves.

To apply a simple visco-elastic mathematical model, the Kelvin-Hooke model, on the curves obtained to calculate the spring and dashpot constants and establish its ability as a first approach for a sponge-in-glycerol model system *in vitro* to explain its mechanical behaviour, so as to apply it to similar displacement versus time curves obtained *in vivo* (Study III).

3.2.3 Methodology

To test the tissue tonometer *in vitro*, a sponge-in-glycerol model was used. The natural sponge was cut into small sections of 1cm by 1cm square and soaked in 0%, 25%, 50% and 100% concentrations of glycerol in a glass beaker with water used as the dilution

medium. The tonometer was calibrated prior to each measurement as described in section 2.8. The plunger with a weight of 30g attached to a 4mm diameter plunger was positioned just above the centre of the sponge with a known concentration of glycerol. The weight was released by means of a cable release mechanism and allowed to sink into the sponge under gravity. The movement of the plunger was recorded for 5 minutes after which, the plunger and weight were released from the sponge. The tonometer was re-calibrated after the measurements and if there was a significant difference between the pre- and post-calibration figures, the test was repeated. The same procedure was repeated on all the different concentrations of glycerol soaked sponges with the tonometer calibrated before each measurement. Three tissue tonometry measurements were recorded for 5 minutes for each % concentration of glycerol and the mean value calculated for analysis.

Data Analysis

The displacement versus time curves obtained from the sponge in glycerol model were analysed using the computer software to calculate the distance and rate constant parameters for the different concentrations of glycerol.

The Kelvin-Hooke spring-dashpot visco-elastic model was applied to analyse the spring and dashpot constants from the traces obtained with the tissue tonometer.

Regression analysis was applied to show the correlation coefficient between the distance and rate constant parameters obtained from the tissue tonometer and the spring and dashpot constants obtained from the Kelvin-Hooke model with increasing viscosity.

3.2.4 Results

Displacement versus time curves obtained from sponge-in-glycerol model

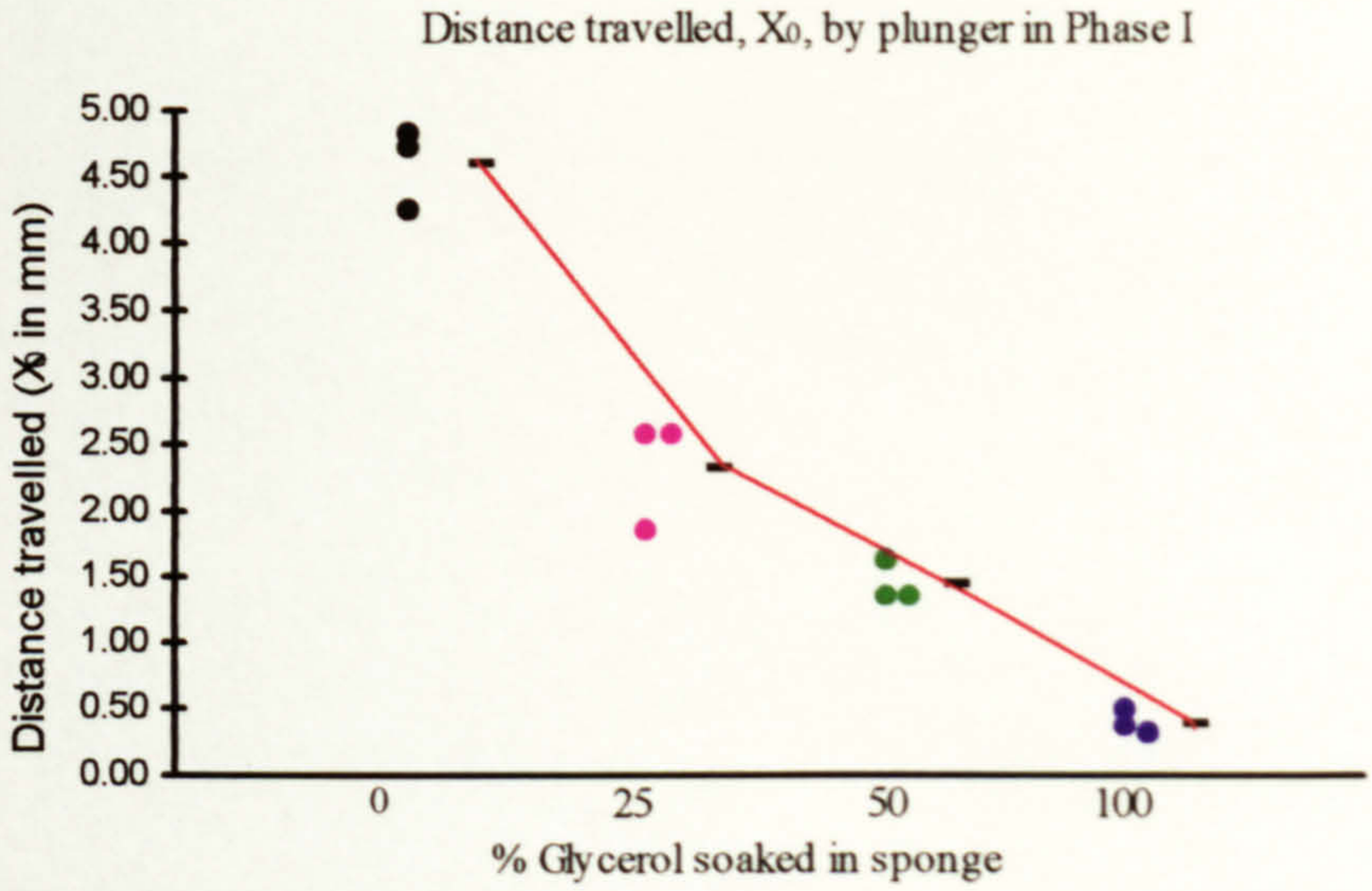


Figure 3.2.1 The mean value of the distance travelled by plunger in the initial fast indentation phase, Phase I, plotted against increased viscosity .

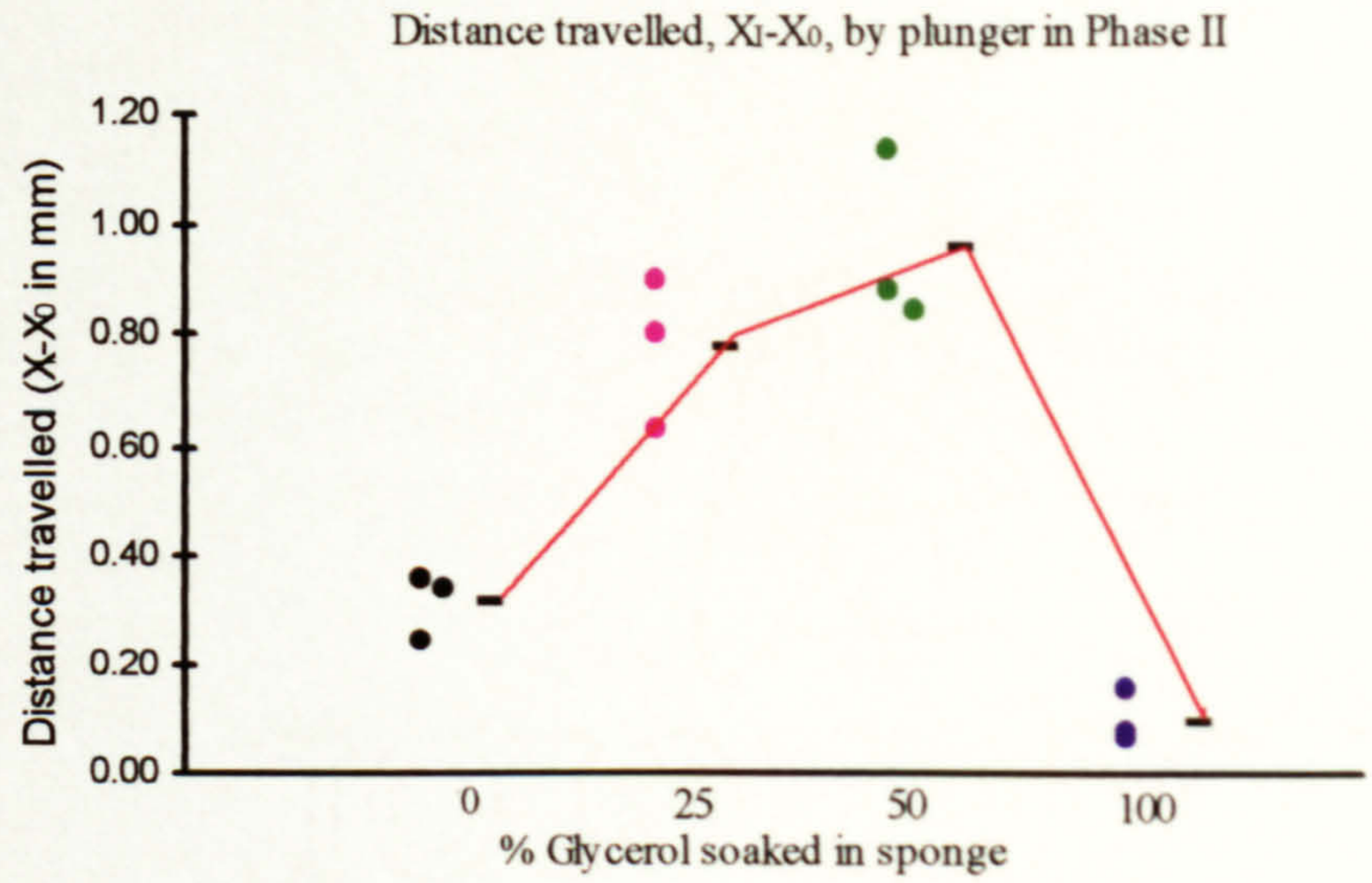


Figure 3.2.2 The mean value of the distance travelled by plunger in the subsequent slower indentation phase, Phase II, plotted against increased viscosity.

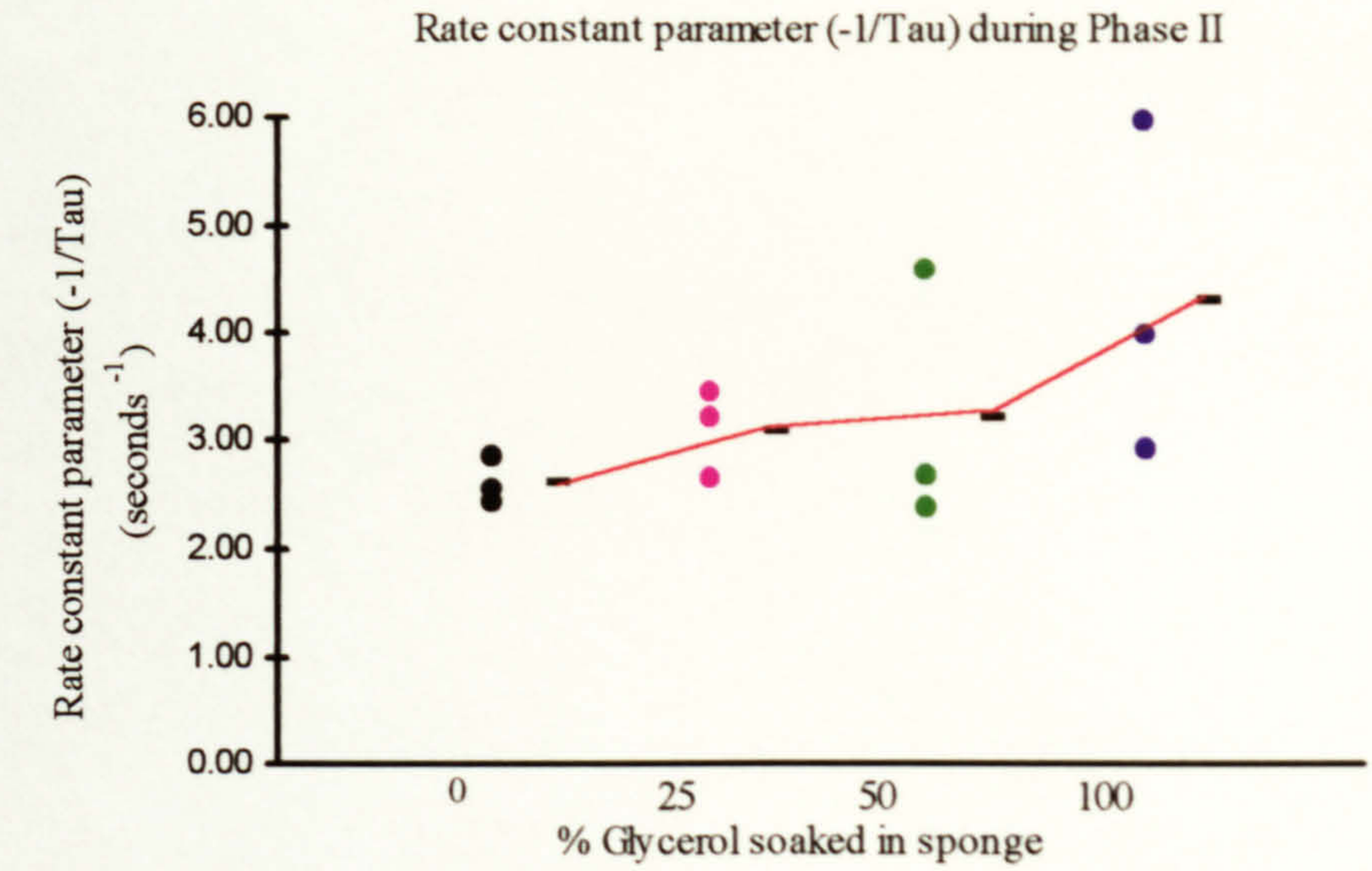


Figure 3.2.3 The mean value of the rate constant parameter in the subsequent slower indentation phase, Phase II, plotted against increased viscosity.

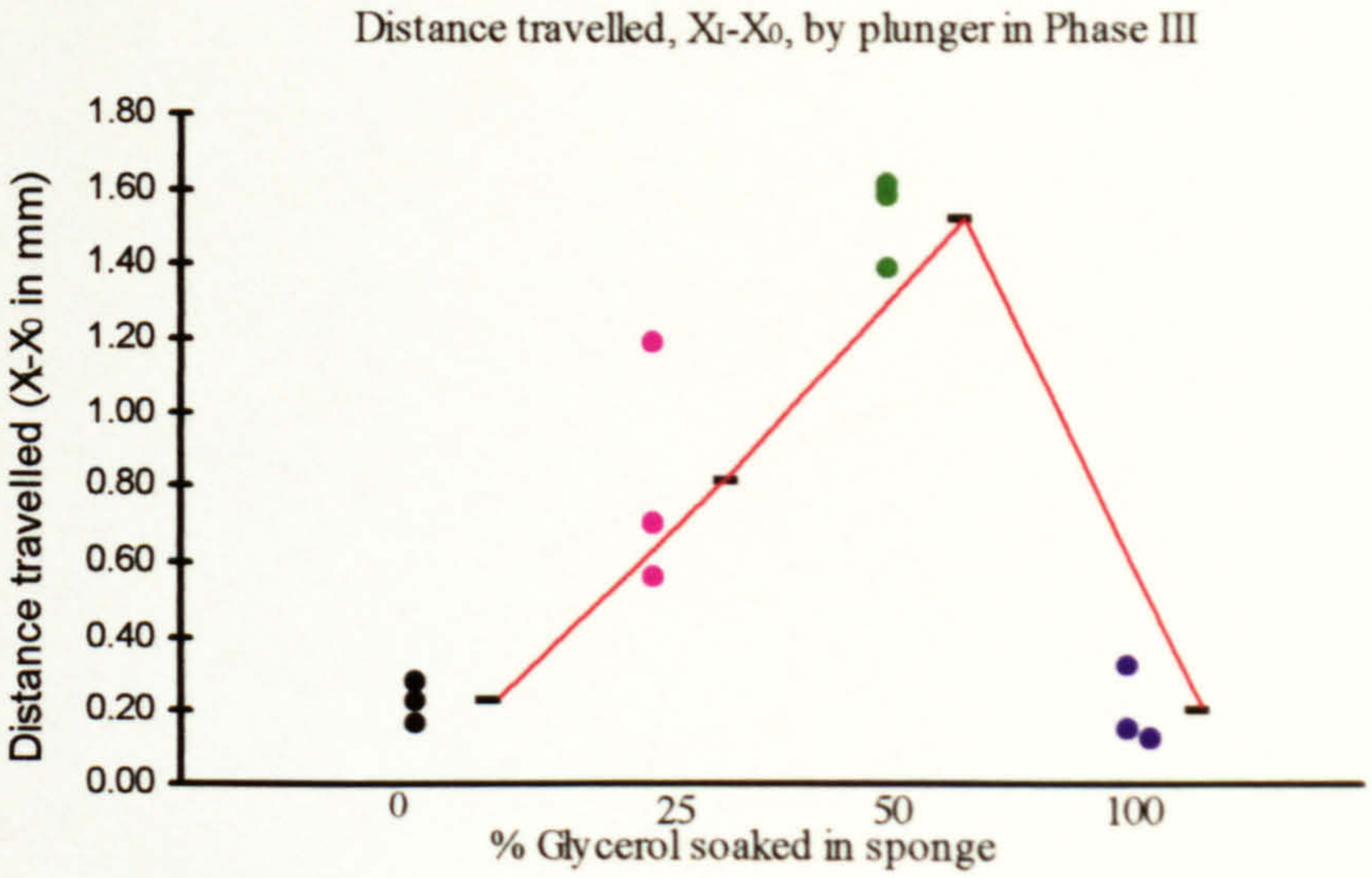


Figure 3.2.4 The mean value of the distance travelled by plunger in the subsequent slower indentation phase, Phase III, plotted against increased viscosity.

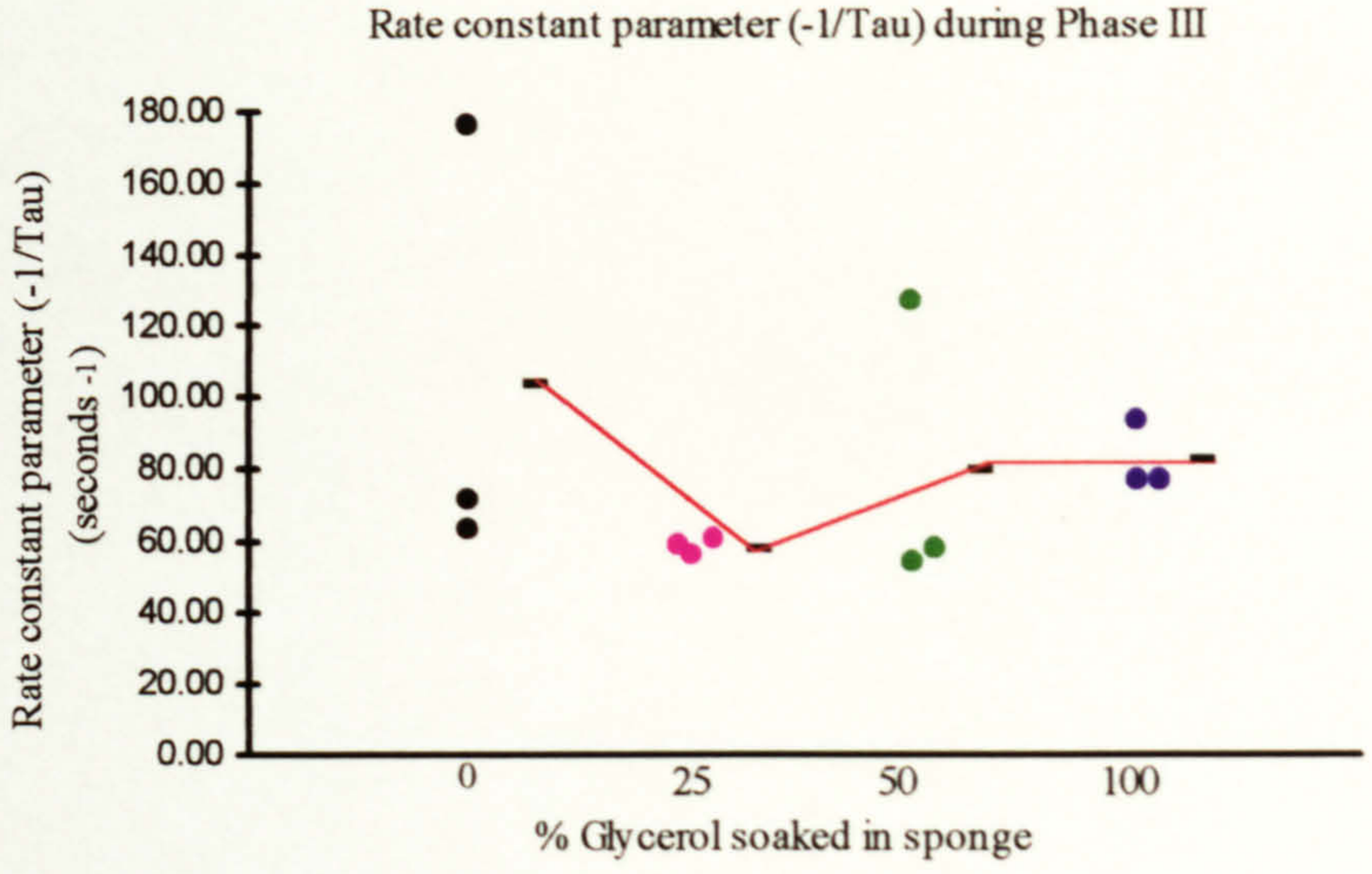


Figure 3.2.5 The mean value of the rate constant parameter in the subsequent slower indentation phase, Phase III, plotted against increased viscosity.

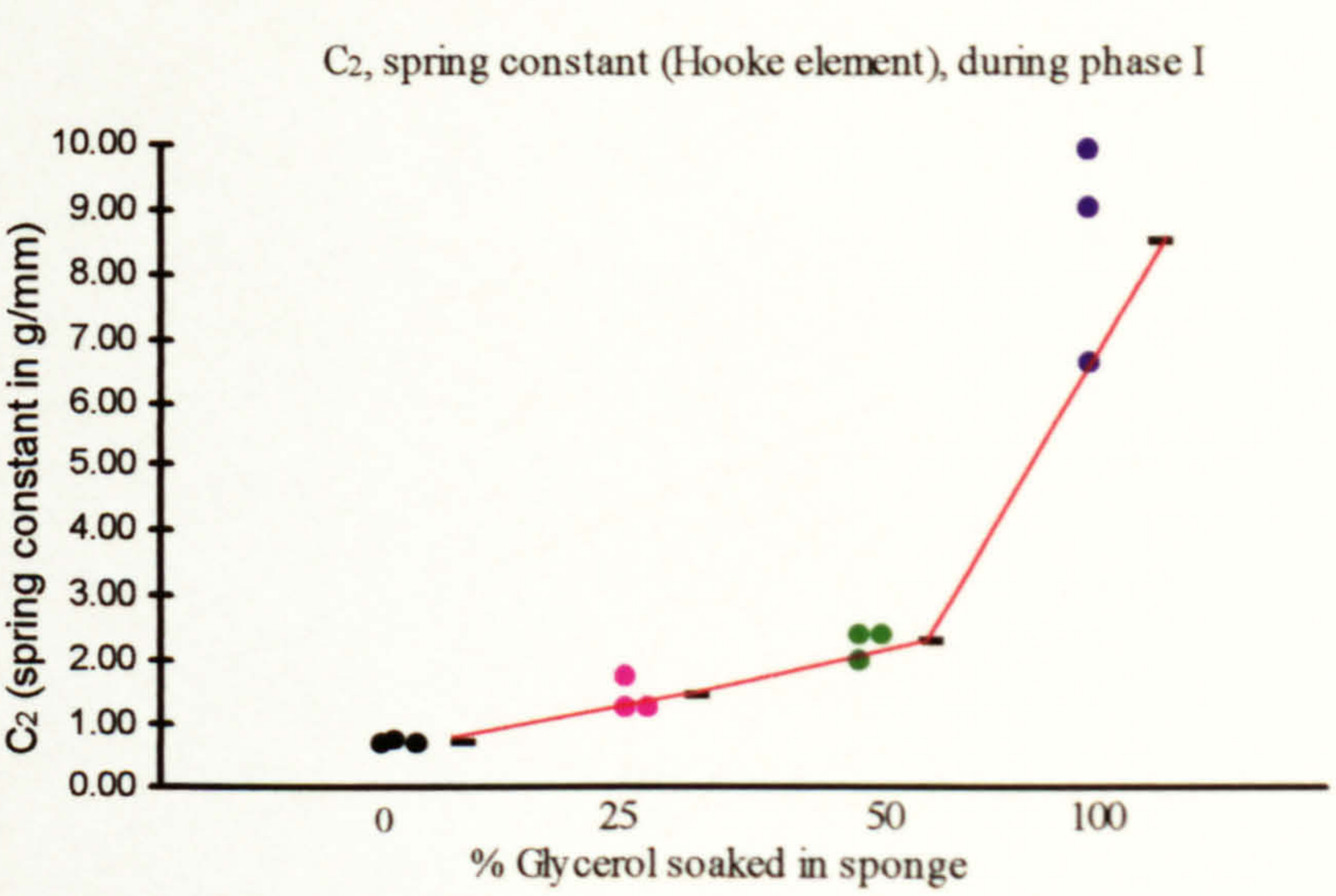


Figure 3.2.6 The mean value of the spring constant, C_2, in the initial fast indentation phase, Phase I, plotted against increased viscosity.

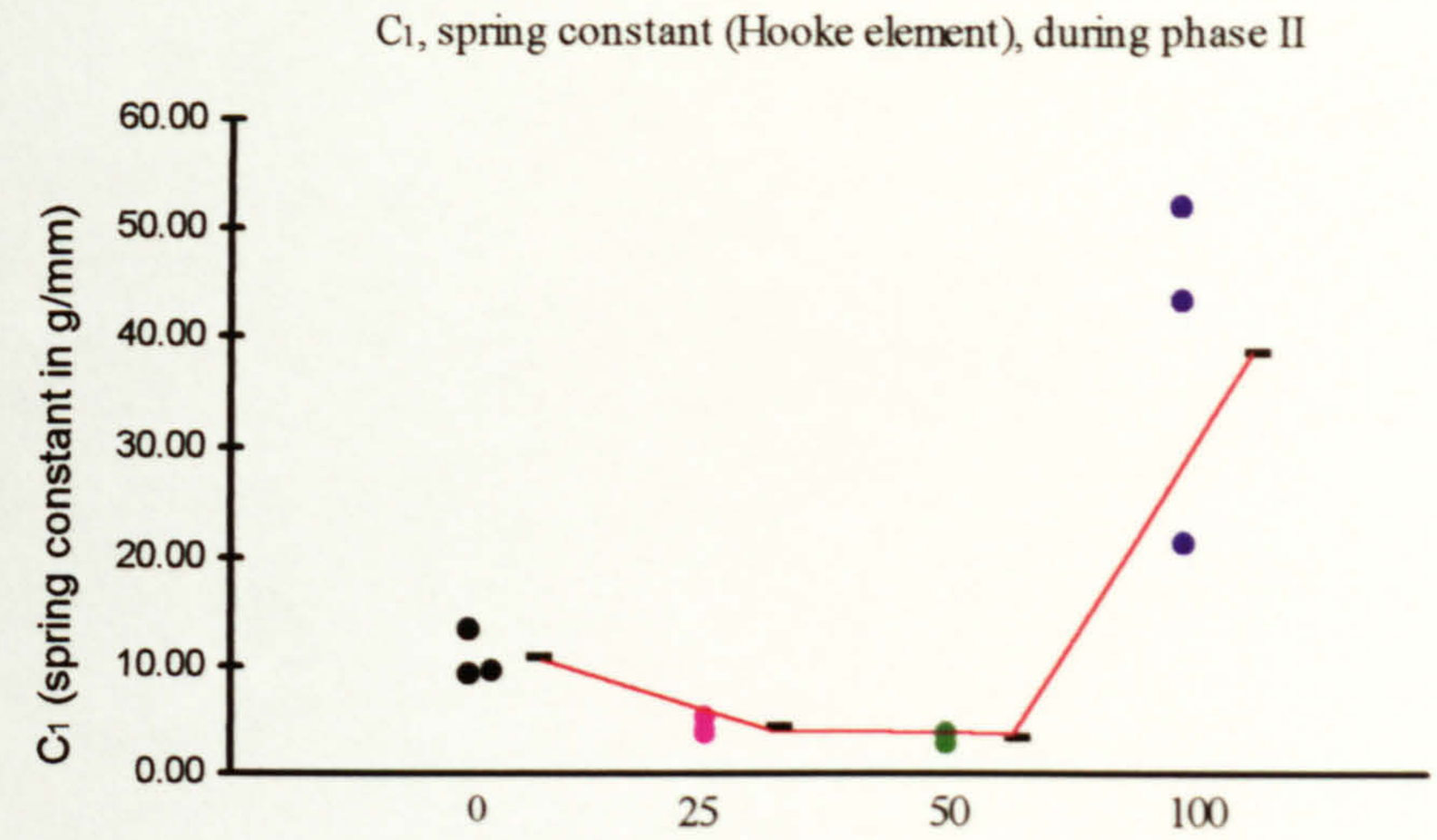


Figure 3.2.7 The mean value of the spring constant, C_1, in the subsequent slower indentation phase, Phase II, plotted against increased viscosity.

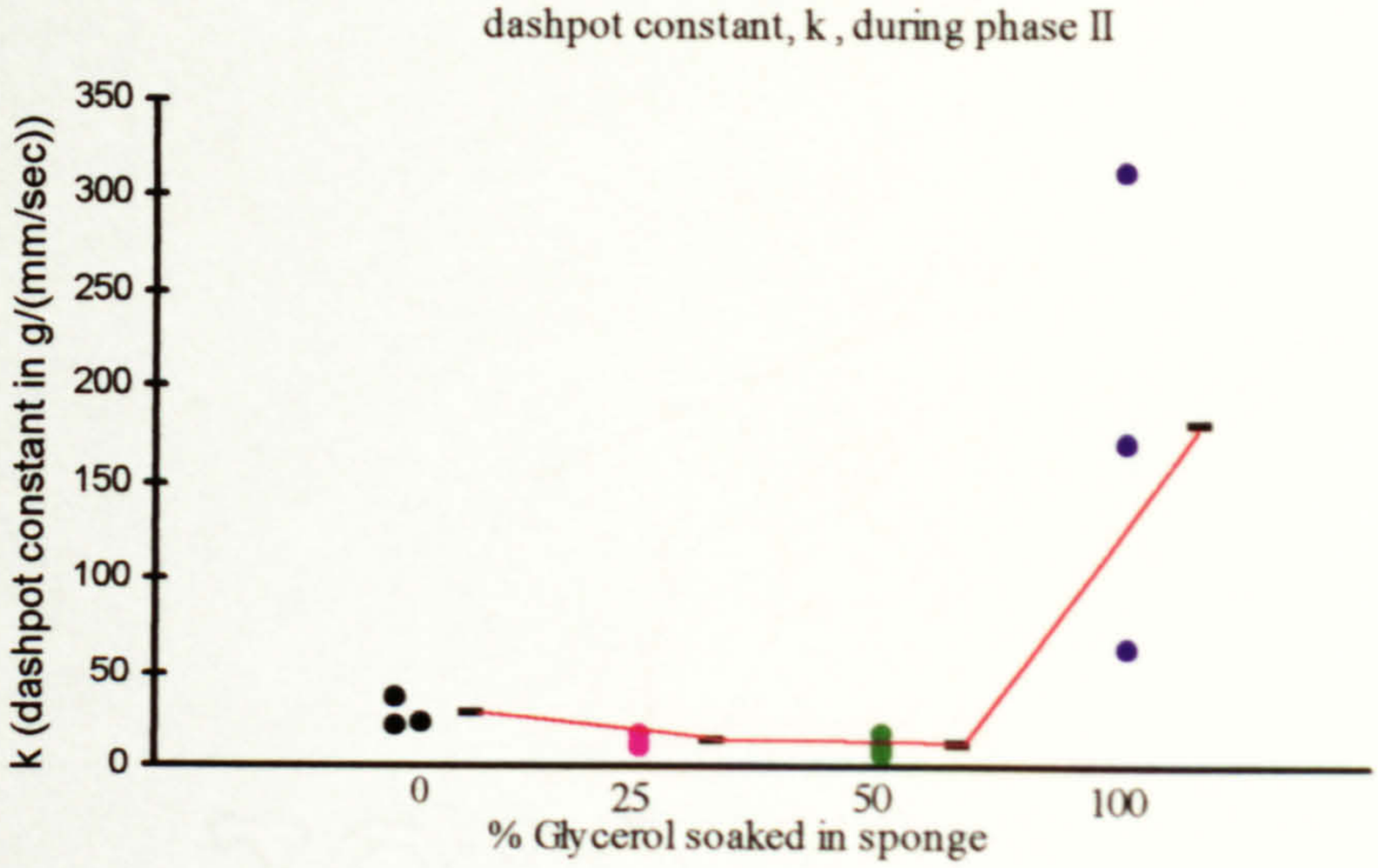


Figure 3.2.8 The mean value of the dashpot constant, k, in the subsequent slower indentation phase, Phase II, plotted against increased viscosity.

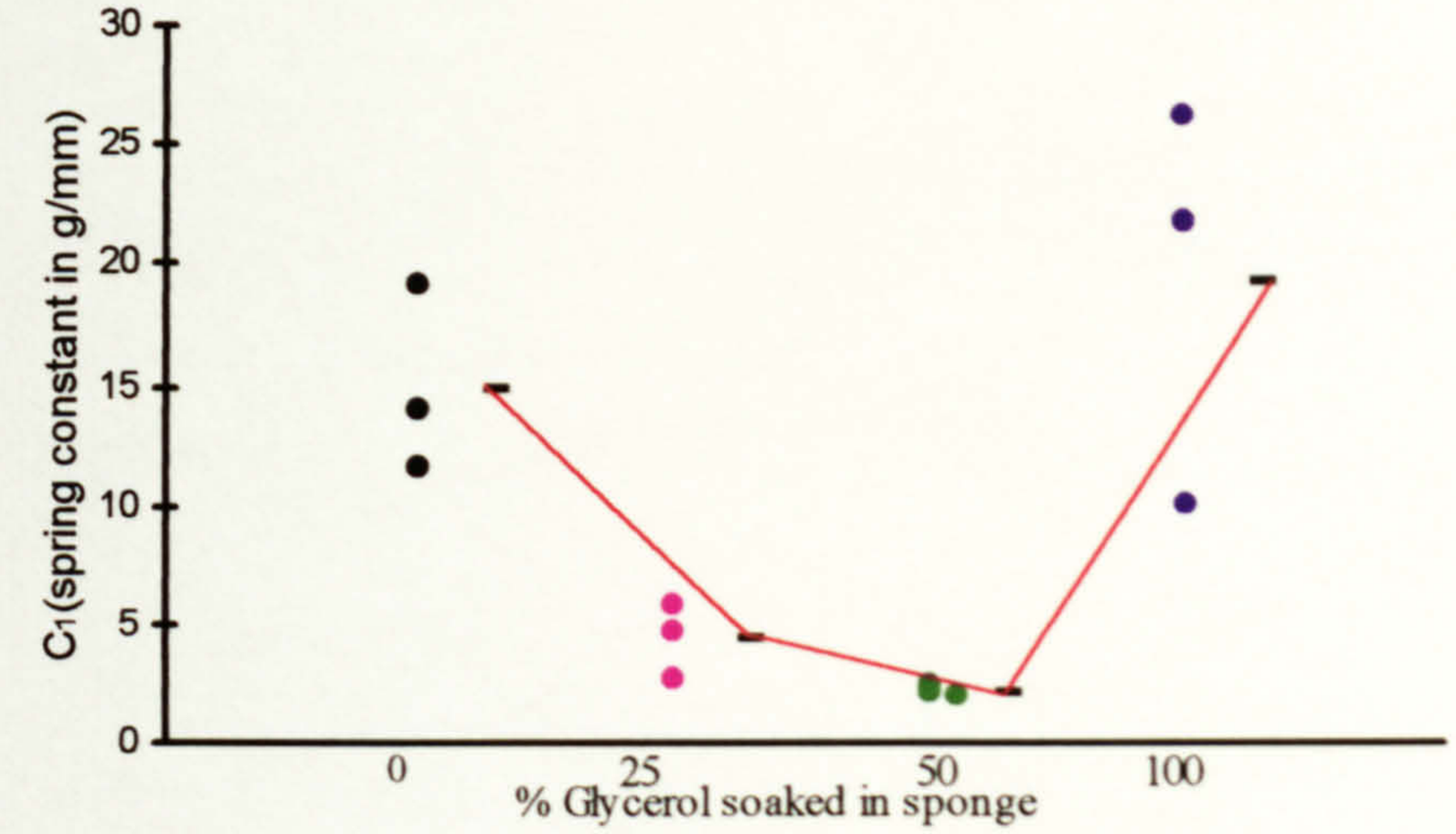


Figure 3.2.9 The mean value of the spring constant, C_1, in the subsequent slower indentation phase, Phase III, plotted against increased viscosity.

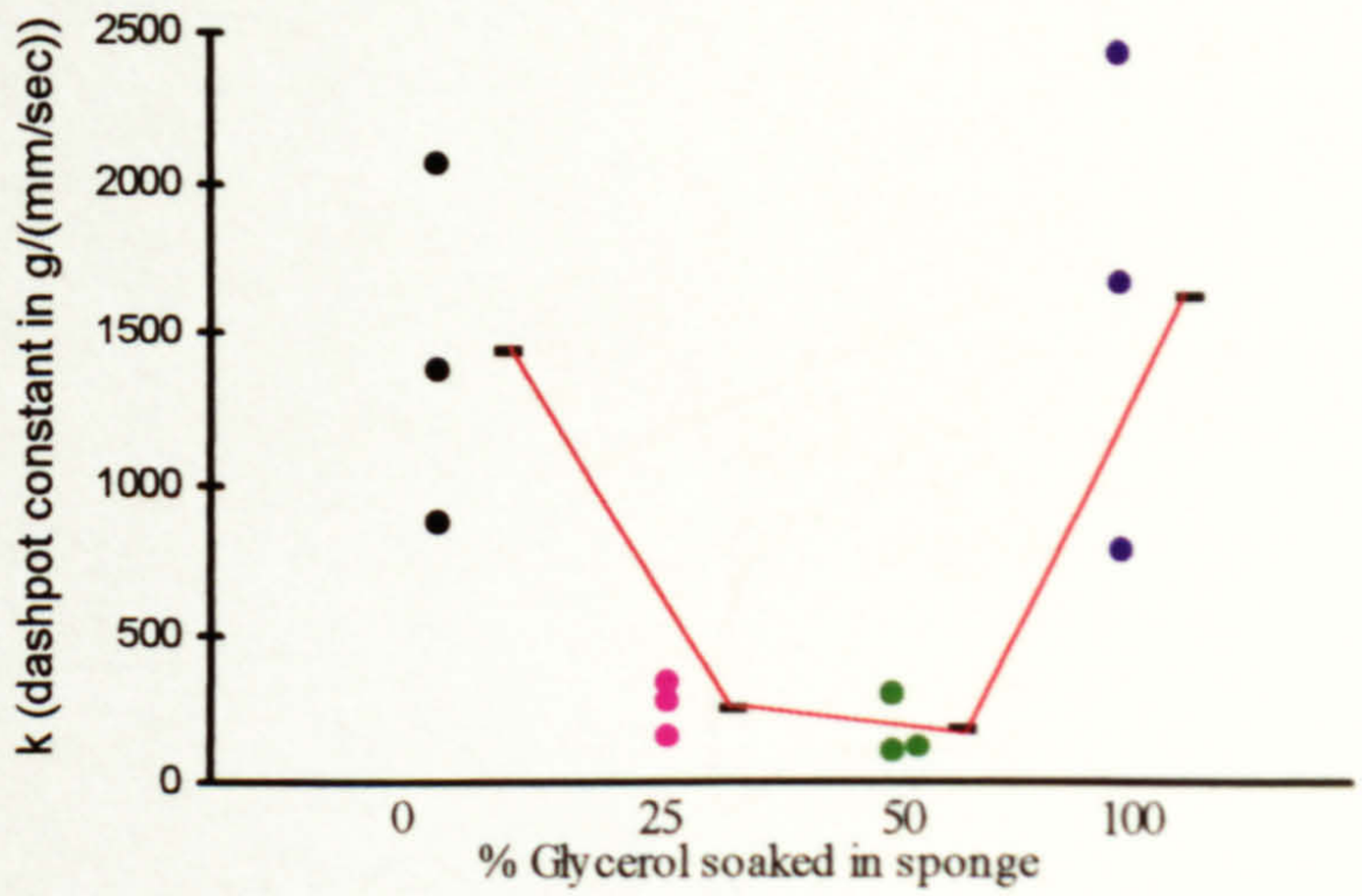


Figure 3.2.10 The mean value of the dashpot constant, k, in the subsequent slower indentation phase, Phase III, plotted against increased viscosity.

Tissue tonometer parameter / phase	r value	Glycerol concentration range
X_0 parameter during Phase I	-0.93	0%,25%,50% & 100%
X_I-X_0 parameter during Phase II	-0.33	0%,25%,50% & 100%
X_I-X_0 parameter during Phase II	0.97	0%,25%,50%
Rate Constant parameter, -1/Tau, during Phase II	0.98	0%,25%,50% & 100%
X_I-X_0 parameter during Phase III	-0.006	0%,25%,50% & 100%
X_I-X_0 parameter during Phase III	1.00	0%,25%,50%
Rate Constant parameter, -1/Tau, during Phase III	-0.21	0%,25%,50% & 100%
Rate Constant parameter, -1/Tau, during Phase III	-0.53	0%,25%,50%
Spring constant, C_2, of the series Hooke element	0.95	0%,25%,50% & 100%
Spring constant, C_1, (Kelvin element) during Phase II	0.78	0%,25%,50% & 100%
Spring constant, C_1, (Kelvin element) during Phase II	-0.91	0%,25%,50%
k, dashpot constant parameter during phase II	0.83	0%,25%,50% & 100%
k, dashpot constant parameter during phase II	-0.91	0%,25%,50%
Spring constant, C_1, (Kelvin element) during Phase III	0.34	0%,25%,50% & 100%
Spring constant, C_1, (Kelvin element) during Phase III	-0.94	0%,25%,50%
k, dashpot constant parameter during phase III	0.25	0%,25%,50% & 100%
k, dashpot constant parameter during phase III	-0.89	0%,25%,50%

Table 3.2.1 Correlation coefficient (r values) obtained from regression analysis for each parameter of each phase with varying glycerol concentration.

The plunger travelled the furthest (X_0 in mm), during phase I, in the 0% glycerol soaked sponge and least in the 100% glycerol soaked sponge with a linear decrease between the 0% and 100% glycerol soaked sponges (figure 3.2.1).

During phase II, the distance travelled by the plunger (X_I-X_0 in mm) increased linearly from 0% to 50% glycerol concentration but interestingly at 100% glycerol concentration, the plunger travelled significantly less into the tissues (figure 3.2.2).

The rate constant parameter during phase II, gradually increased as the viscosity of the glycerol increased but the rise was not significant (figure 3.2.3).

During phase III, the distance travelled by the plunger (X_I-X_0 in mm), the pattern of sinking of the plunger into the sponge was similar to that of phase II with the distance

travelled by the plunger increasing linearly from 0% to 50% glycerol but was significantly reduced in the 100% glycerol (figure 3.2.4).

The rate constant parameter during phase III, was higher in the 0% glycerol but there was no significant change in the 25%, 50% and 100% glycerol (figure 3.2.5) groups.

The initial rapid deformation of a visco-elastic material as explained by the Kelvin-Hooke model depended on its C_2 elastic spring constant or Hooke element. In figure 3.2.6, there was a linear increase in the C_2 elastic spring constant with increasing viscosity from 0% to 100% glycerol soaked sponges. During phase I, there was a decrease in the C_2 value as the viscosity increased with an increase in the final value of $X_I - X_0$.

C_1, the spring constant of the series Kelvin element, decreases as the final distance travelled by the plunger increases. In figures 3.2.7 and 3.2.9, the C_1 spring constant in phases II and III decreased between the 0%, 25% and 50% glycerol soaked sponges as the distance travelled by the plunger increased between these concentrations. C_1 increased quite significantly between the 50% and the 100% glycerol soaked-sponges as the distance travelled by the plunger was significantly reduced at the 100% glycerol concentration (figures 3.2.2 and 3.2.4).

During phases II and III, the distance travelled by the plunger increased (figures 3.2.2 and 3.2.4) and the dashpot value, k, decreased from 0% to 50% glycerol concentration (figures 3.2.8 and 3.2.10). The dashpot constant, k, increased quite significantly between 50% and 100% glycerol concentration.

Regression analysis was done to calculate the correlation coefficient between the tissue tonometry parameters and glycerol concentration (see table 3.2.1)

There was very good negative correlation between the distance travelled X_0 (mm) during phase I and the increase in the viscosity of the glycerol ($r = -0.93$).

There was poor correlation between the distance travelled, $X_I - X_0$, and the increase in glycerol viscosity from 0% to 100%, during the slower indentation phase (phase II) ($r = -0.33$). However, the distance travelled by the plunger increased linearly with the increase in glycerol viscosity from 0% to 50% glycerol with a very good correlation ($r=0.97$). This same trend is repeated for the distance travelled, $X_I - X_0$ during phase III ($r=-0.06$ and $r=1.00$).

The Kelvin-Hooke model was applied to see if the tissue tonometry readings would fit the model. The spring constant C_2 increased linearly with the increase in glycerol concentration ($r=0.95$).

There was moderate correlation between the Kelvin spring constant C_1 and increasing viscosity (r=0.78) from 0% to 100% during phase II. However, with the removal of the 100% glycerol, this was the case with resulting good correlation (r= - 0.91) as C_1 decreases the distance travelled by the plunger increased (Figure 3.2.7).

This pattern was the same for the dashpot constant, k, during phase II and for the C_1 and k parameters during phase III (see table 3.2.1).

3.2.5 Discussion

This study was under taken to assess the performance of the tonometer system *in vitro*. There was no attempt to simulate the real mechanical properties of the skin and subcutaneous tissues with this model, and the findings differ in a number of respects from those which were obtained in volunteers and patients.

During the initial fast indentation phase I, the plunger travelled the furthest in the 0% glycerol soaked sponge and least in the 100% glycerol soaked sponge with a linear decrease in the distance travelled (X_0 in mm) by the plunger between the 0% and 100% glycerol soaked sponge (figure 3.2.1) during phase I. This phase reflects the fall in compliance of the sponge and with the increasing viscosity of the glycerol. This was possibly because as the cells within the sponge became filled with fluid of increasing viscosity, it increased the resistive forces to plunger travel.

During phase II, the slower indentation phase between 1 and 10 seconds, the distance travelled by the plunger (X_I-X_0 in mm) increased linearly from 0% to 50% glycerol concentration but interestingly at 100% glycerol concentration, the plunger travelled significantly less into the tissues (figure 3.2.2). This suggests that the solvent used (water) to dilute the glycerol may have had a softening effect on the sponge.

During phase III, the distance travelled by the plunger (X_I-X_0 in mm) in the slower indentation phase lasting about 290 seconds, the pattern of sinking of the plunger into the sponge was similar to that of phase II with the distance travelled by the plunger increasing linearly from 0% to 50% glycerol but was significantly reduced in the 100% glycerol (figure 3.2.4) again possibly due to the reasons explained in phase II distance parameter above. The rate constant parameter during phase II, gradually increased as the viscosity of the glycerol increased but the rise was not significant (figure 3.2.3).

The rate constant parameter during phase III, was higher in the 0% glycerol but there was no significant change in the 25%, 50% and 100% glycerol (figure 3.2.5) groups. However, a reduced rate constant means that the final distance travelled by the plunger

was reached more slowly. Therefore, the plunger sank into the 25%, 50% and 100% glycerol soaked sponges for longer as compared to the 0% glycerol sponge in which the resistive forces of the dilution medium (water) to the plunger within the sponge cells were less than those of the glycerol which is more viscous and thus provides a greater resistive force.

The initial rapid deformation of a visco-elastic material as explained by the Kelvin-Hooke model depended on its C_2 elastic spring constant or Hooke element. It represents the initial ability of the interstitial matrix to undergo elastic deformation without flow. In figure 3.2.6, there was a linear increase in the C_2 elastic spring constant with increasing viscosity from 0% to 100% glycerol soaked sponges.

According to the Kelvin-Hooke model, C_1, the spring constant of the series Kelvin element, is inversely related to the final distance travelled by the plunger. An increase in the final value that the plunger reaches (X_1 - X_0) is as a result of a decrease in C_1. In figures 3.2.7 and 3.2.9, the C_1 spring constant in phases II and III decreased between the 0%, 25% and 50% glycerol soaked sponges as the distance travelled by the plunger increased between these concentrations. C_1 increased quite significantly between the 50% and the 100% glycerol soaked-sponges as the distance travelled by the plunger was significantly reduced at the 100% glycerol concentration (figures 3.2.2 and 3.2.4).

According to the Kelvin-Hooke model, the dashpot constant, k, is proportional to the resistance to flow. Therefore an increase in the dashpot constant will cause an increase in the time the plunger takes to level off. During phases II and III (figure 3.2.8 and 3.2.10), the distance travelled by the plunger increased (figures 3.2.2 and 3.2.4) and the dashpot value, k, decreased from 0% to 50% glycerol concentration. The dashpot constant, k, increased quite significantly between 50% and 100% glycerol concentration suggesting a softening effect of the solvent on the sponge. In this model, the k value is lower at 50% glycerol than at 0% glycerol but is higher at 100% glycerol than at 50% glycerol suggesting that this parameter is less sensitive at lower viscosities than at higher viscosities.

There was very good negative correlation between the distance travelled X_0 (mm) during phase I and the increase in the viscosity of the glycerol ($r = -0.93$)(table 3.2.1). As the glycerol viscosity increased, the sponge compliance which is reflected by the X_0 distance parameter linearly decreased. According to the Kelvin-Hooke model, the X_0 parameter should depend only on the initial elastic compressibility and not on the viscosity of the fluid in the matrix. This clearly was not the case in the sponge in

glycerol model which could be attributed to the mechanical properties of the sponge. This is composed of cells filled with air initially and when it is soaked in the glycerol solution, these air cells become filled with the glycerol and as the viscosity of the glycerol solution is increased the resistance in these cells increases thus providing a greater resistance to the plunger as it sinks into the tissues. This change of resistance in the cells of the sponge tissue probably affect the mechanical properties. The greater the resistance, the less the distance travelled by the plunger. In another study on the sponge in glycerol model with a tissue tonometer (Bates et al, 1994), it was found that the distance travelled X_0 was not significantly different between 25%, 50% and 75% concentration of glycerol soaked sponges but was significantly reduced in the 100% glycerol concentration. The weight used on the plunger in his study was significantly higher (152g) than the weight applied in this study (30g). It is possible that the weight applied in the Bates' study was too heavy and compressed the sponge rapidly in the 25%, 50% and 75% glycerol concentration. This was demonstrated in study I table 3.1.4 as I tried different weights and plunger diameters and found that the X_0 parameter generally increased with increasing weights for the same sponge in glycerol model.

There was poor correlation between the distance travelled, X_I-X_0, and the increase in glycerol viscosity from 0% to 100%, during the slower indentation phase (phase II) (r = -0.33)(table 3.2.1). However, the distance travelled by the plunger increased linearly with the increase in glycerol viscosity from 0% to 50% glycerol with a very good correlation (r=0.97) (table 3.2.1). A possible explanation for this is that the 100% glycerol solution provided too great a resistance within the open cells of the sponge for the 30g weight applied to counteract and hence the distance travelled is less than in the other concentrations. This same trend is repeated during phase III probably for the same reasons as above (table 3.2.1).

For the 30g weight applied to the 100% glycerol concentration model, the distance and rate constant parameters calculated by the tissue tonometer was not suitable proving too high a resistance for it to counteract. The 0%, 25% and 50% sponge in glycerol model appeared to have a very good correlation.

The Kelvin-Hooke model was applied to see if the tissue tonometry readings would fit the model. From the above model, the X_0 parameter and the elastic spring constant of the Hooke element, C_2, which provides a measure of the tissue compliance, should depend only on the initial elastic compressibility and not on the viscosity of the fluid in the matrix. The spring constant C_2 increased linearly with the increase in glycerol

concentration (r=0.95) (table 3.2.1) which was similar to the values for the X_0 parameter above. This is not surprising as C_2 is inversely related to X_0.

There was moderate correlation between the Kelvin spring constant C_1 and increasing viscosity (r=0.78; table 3.2.1) from 0% to 100% during phase II. This was not as the Kelvin-Hooke model predicted because as the value of C_1 decreases the final distance travelled by the plunger should increase. However, with the removal of the 100% glycerol, this was the case with resulting good correlation (r= - 0.91) as C_1 decreases the distance travelled by the plunger increased (table 3.2. 1).

This pattern was the same for the dashpot constant, k, during phase II (table 3.2.1). This fits with the Kelvin-Hooke model because k is expected to be lower as the final distance travelled by the plunger increased (Figure 3.2.2).

The same pattern is repeated for the C_1 and k parameters during phase III (table 3.2.1; figure 3.2.4)

3.2.6 Conclusion

In the sponge in glycerol model, the application of the Kelvin-Hooke model appeared to fit the data and this model would now be applied *in vivo* to analyse the mechanical properties of the skin of patients with lower limb chronic venous disease using a tissue tonometer.

3.3 STUDY III

Use of a tissue tonometer *in vivo* to assess the skin changes of patients with lower limb chronic venous disease and the application of a simple mathematical model in the analysis of the displacement versus time curves obtained to explain its mechanical behaviour.

3.3.1 Rationale

In study I, I have shown that patients with liposclerotic skin changes with no palpable induration (C_{4a})should be differentiated from patients with liposclerotic skin changes and palpable induration (C_{4b}) as a result of the significant differences in the skin compliance between the two groups ($p<0.0005$) (Figure 3.1.9; Table 3.1.1). The CEAP method of classification does not differentiate between these two groups as they are both included in the C_4 group. I have proposed separating these two groups into C_{4a} and C_{4b} respectively based on the evidence provided by the mechanical properties of the skin compliance as measured by the tissue tonometer.

The Kelvin-Hooke model has been previously applied (Bates et al, 1994) to displacement versus time curves obtained with a tissue tonometer to measure post-mastectomy lymphoedematous arms and was shown to fit the tonometry results remarkably well.

In this study, I will apply the Kelvin-Hooke model, on the resulting displacement versus time curves obtained for all *in vivo* measurements to explain the mechanical properties of the skin changes in patients with lower limb chronic venous disease as compared to normal controls as classified by the CEAP method. This simple mechanical model was applied *in vitro* in study II using a sponge in glycerol model and found that the tissue tonometry findings fitted the data quite well.

3.3.2 Aims

Assessment of skin changes with a tissue tonometer in patients with lower limb chronic venous disease classified according to the CEAP method of classification to obtain displacement versus time curves.

To calculate spring and dashpot constants from the displacement versus time curves obtained by applying the Kelvin-Hooke model to explain the mechanical behaviour of the skin in groups of patients and normal controls.

3.3.3 Methodology

One hundred and eighty one limbs of 102 subjects (56M:46F; mean age of 56.1 years; age range: 20-83 years) with lower limb venous disease demonstrated by duplex ultrasound scanning in the vascular laboratory studied. Patients were classified according to the CEAP method of classification from C_0 to C_6. The CEAP modification was applied here to separate patients with skin changes and no palpable induration, C_{4a}, from those with skin changes with palpable evidence of induration C_{4b}. Resting ABPI were also measured to exclude any arterial disease. The subjects were classified as follows: C_0 (n=20), C_1 (n=26), C_2 (n=17), C_3 (n=25), C_{4a} (n=23), C_{4b} (n=26), C_5 (n=24) and C_6 (n=20).Three different sites were measured in the gaiter area to obtain a mean value as described in section 2.8 at 6cm, 10cm and 12cm above the medial malleolus. If an open ulcer is present at the above sites, the nearest area of skin with no open wound is used and the location noted for measurements on sequential visits. Identical sites were measured on the contralateral limb using the specially calibrated stand with a fixed marker pen (see section 2.5). This procedure was repeated on the other limb using the same heights noted on the calibrated column and the same landmarks to ensure identical sites are marked. With three identical sites marked in both lower limbs, the subjects were then examined by tissue tonometry as described in section 2.8.

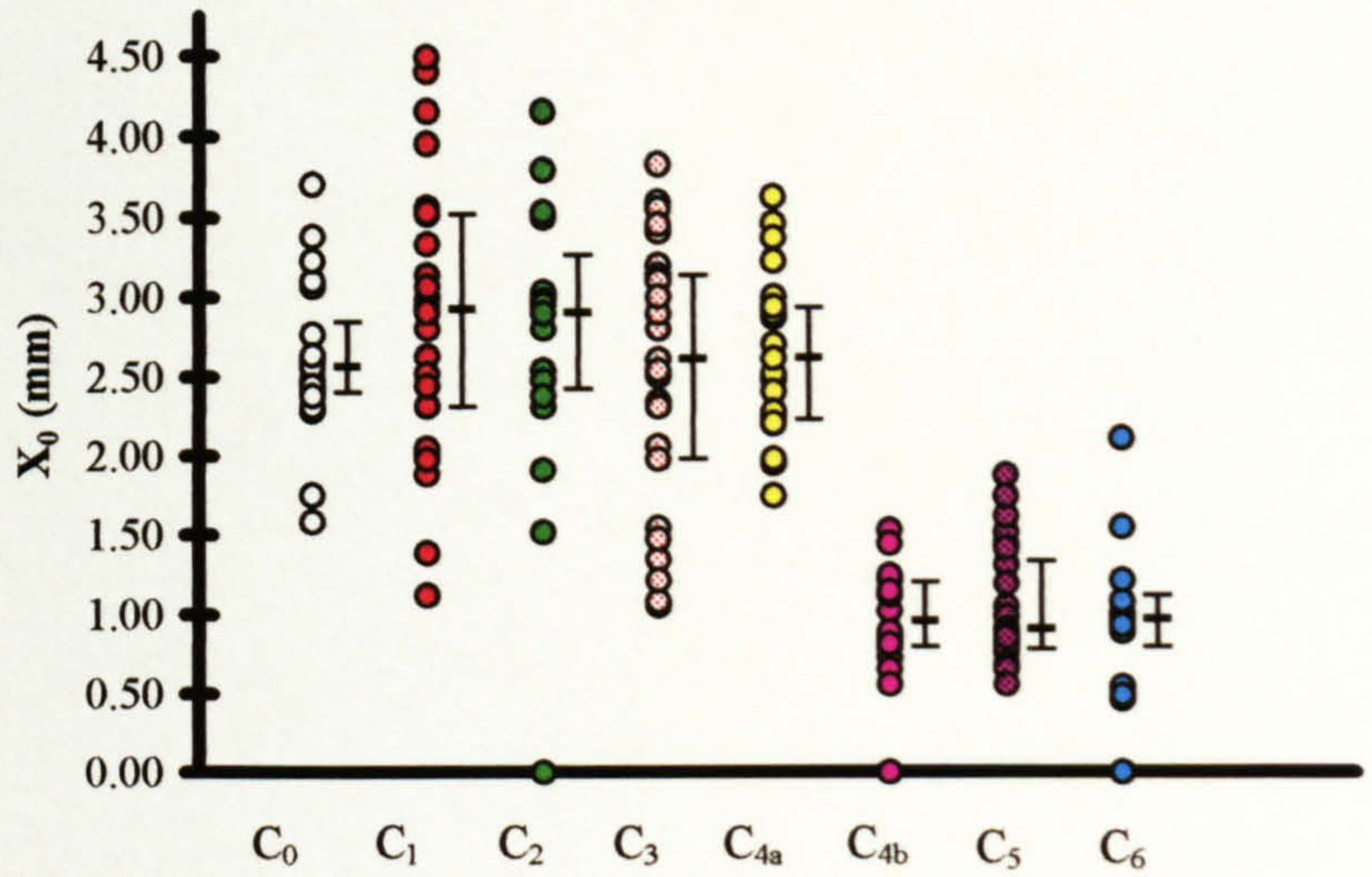
The traces obtained were analysed using the computer software to calculate the distance and rate constant parameters and the spring and dashpot constants as described in the Kelvin-Hooke model.

Data Analysis

The distance and rate constant parameters were plotted as scattergrams for all the different classification groups and medians and interquartile ranges were used as descriptors. The Mann Whitney U test was applied to test for statistical significance.

The spring and dashpot constants were calculated from the Kelvin-Hooke model and plotted as scattergrams with the descriptors and statistical tests used as described above.

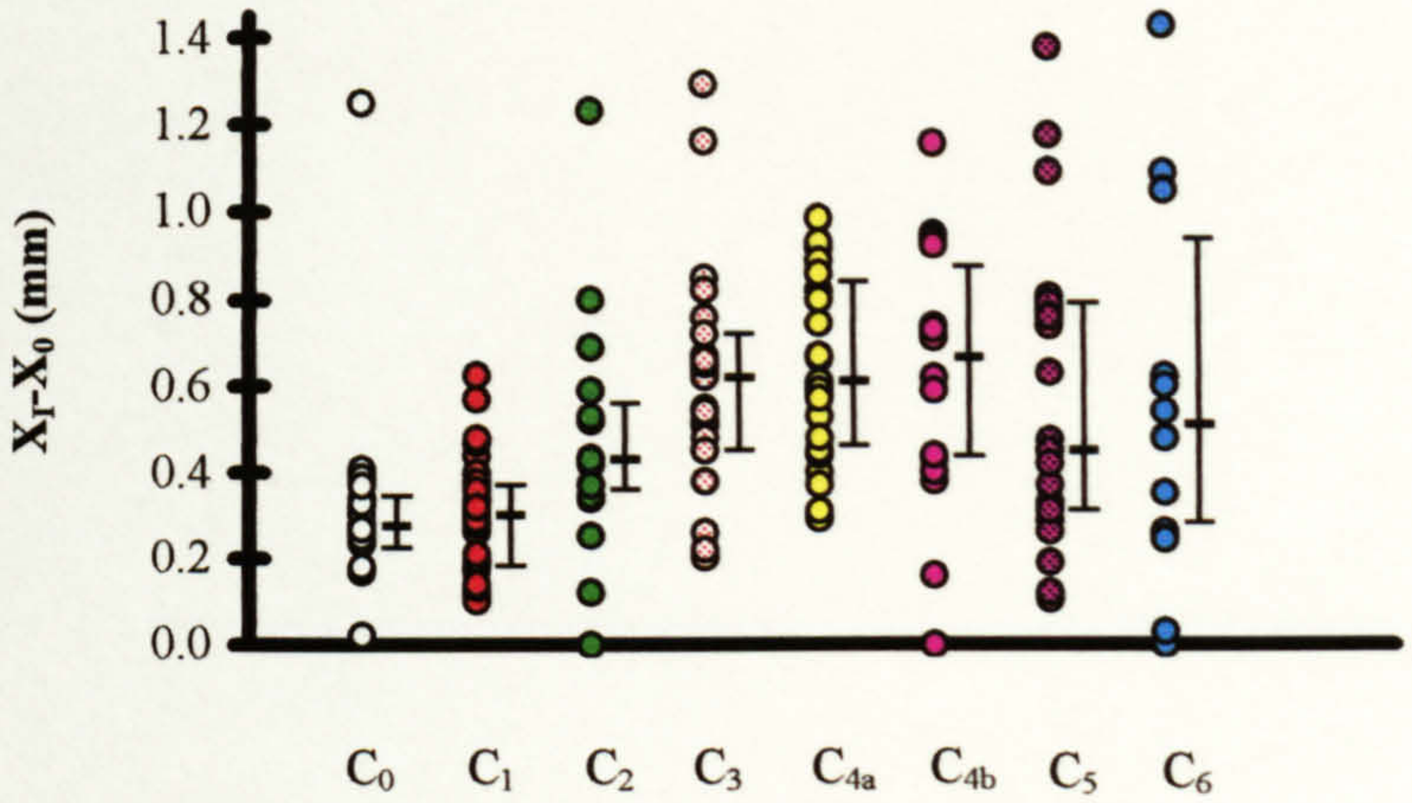
3.3.4. Results



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.42						
C_2	0.79	0.59					
C_3	0.87	0.42	0.73				
C_{4a}	0.97	0.34	0.38	0.95			
C_{4b}	0.0004	0.0002	0.0002	0.0001	0.0001		
C_5	0.0004	0.0002	0.0002	0.0001	0.0001	0.70	
C_6	0.0004	0.0002	0.0002	0.0001	0.0001	0.97	0.82

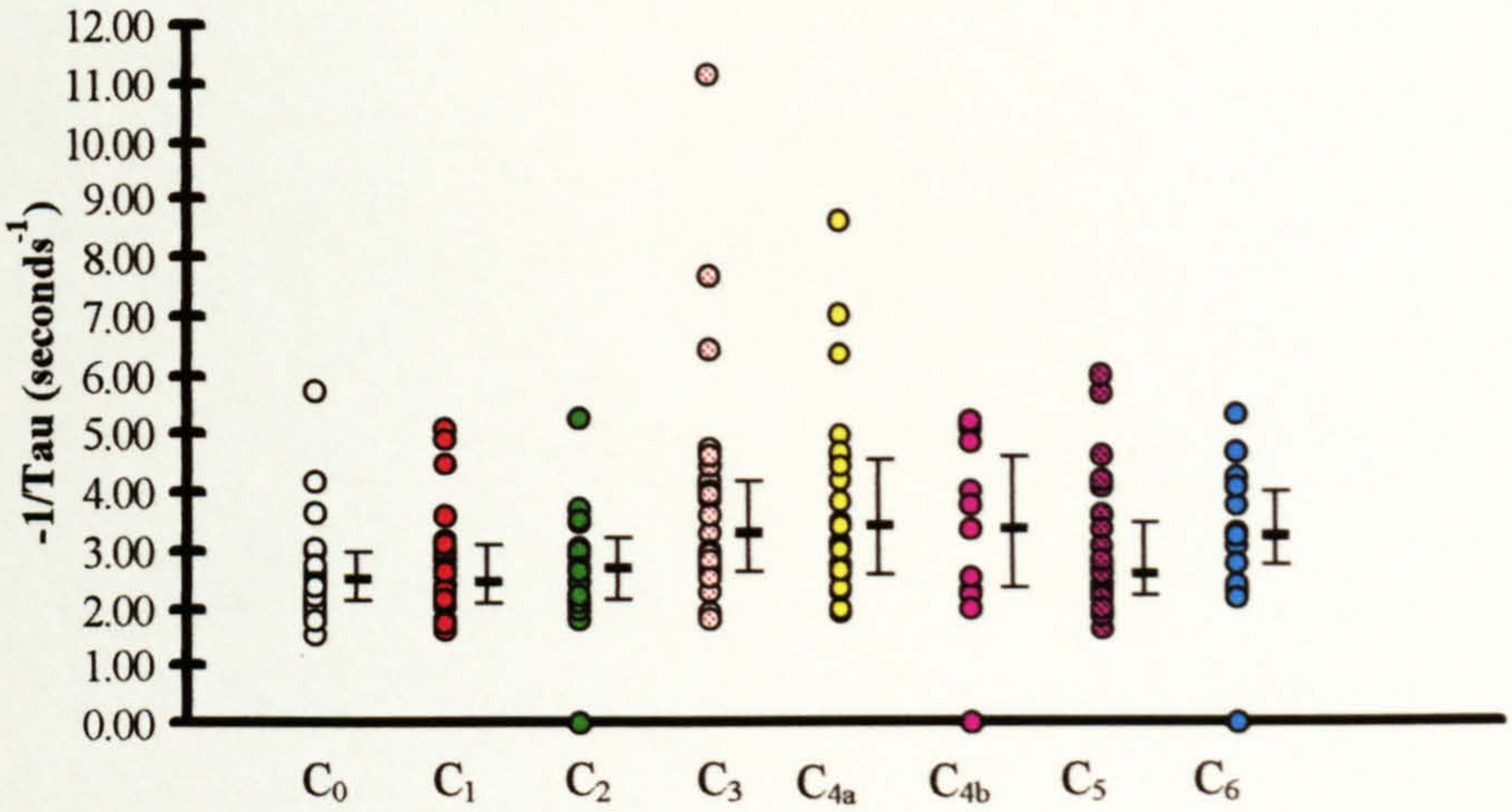
Figure 3.3.1 Distance travelled by plunger (mm) during phase I in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.

Phase II Distance parameter (X_I-X_0) vs CEAP classification



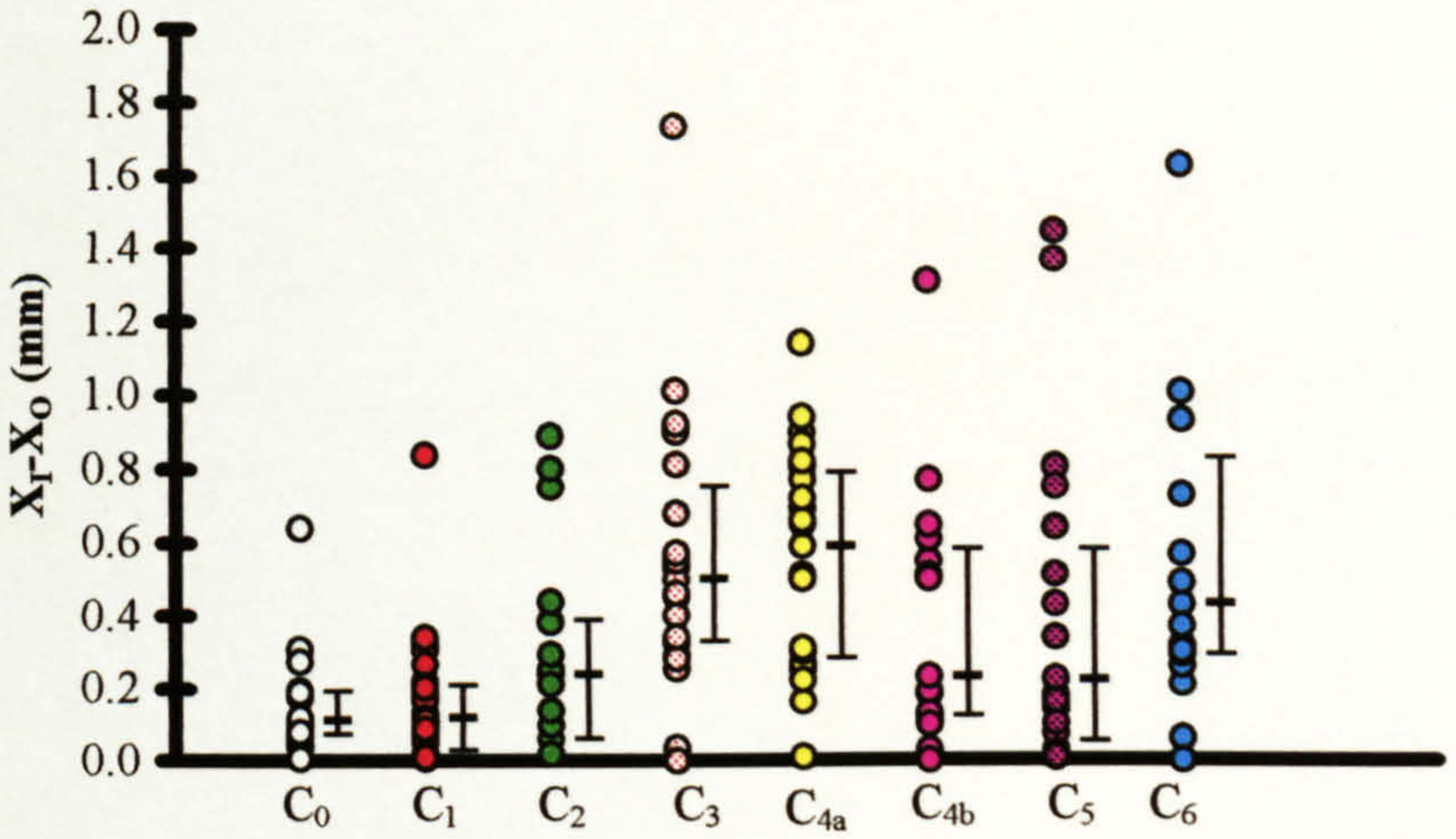
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.50						
C_2	0.002	0.05					
C_3	0.006	0.03	0.65				
C_{4a}	0.0007	0.004	0.21	0.11			
C_{4b}	0.0004	0.001	0.04	0.03	0.26		
C_5	0.02	0.11	0.94	0.97	0.40	0.17	
C_6	0.009	0.03	0.57	0.72	0.66	0.31	0.62

Figure 3.3.2 Distance travelled by plunger (mm) during phase II in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



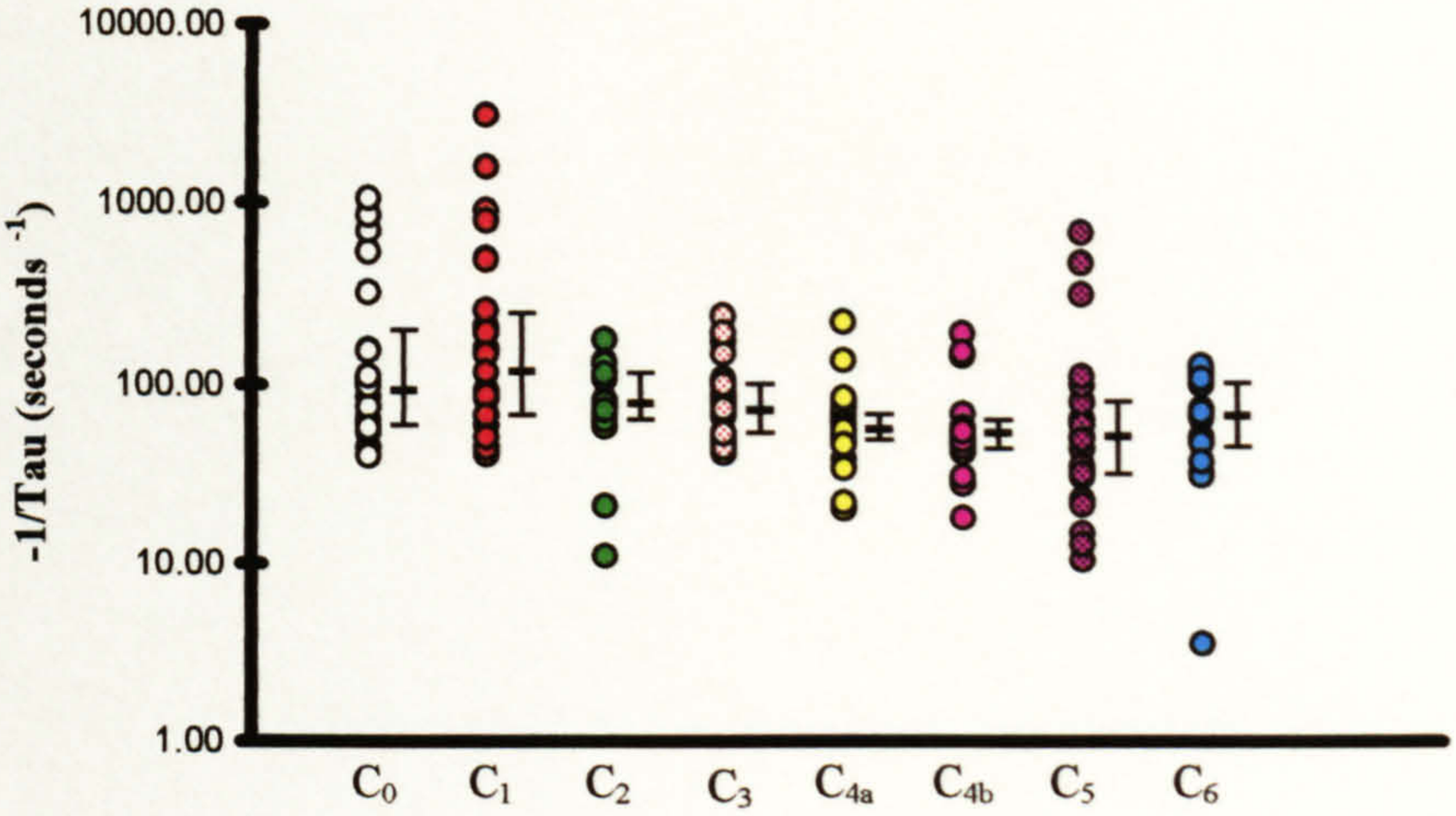
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.93						
C_2	0.65	0.76					
C_3	0.01	0.07	0.14				
C_{4a}	0.03	0.08	0.55	0.88			
C_{4b}	0.21	0.23	0.34	0.89	0.85		
C_5	0.72	0.76	0.94	0.10	0.15	0.34	
C_6	0.06	0.09	0.23	0.53	0.64	0.85	0.36

Figure 3.3.3 Rate constant parameter (secs $^{-1}$) during phase II in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



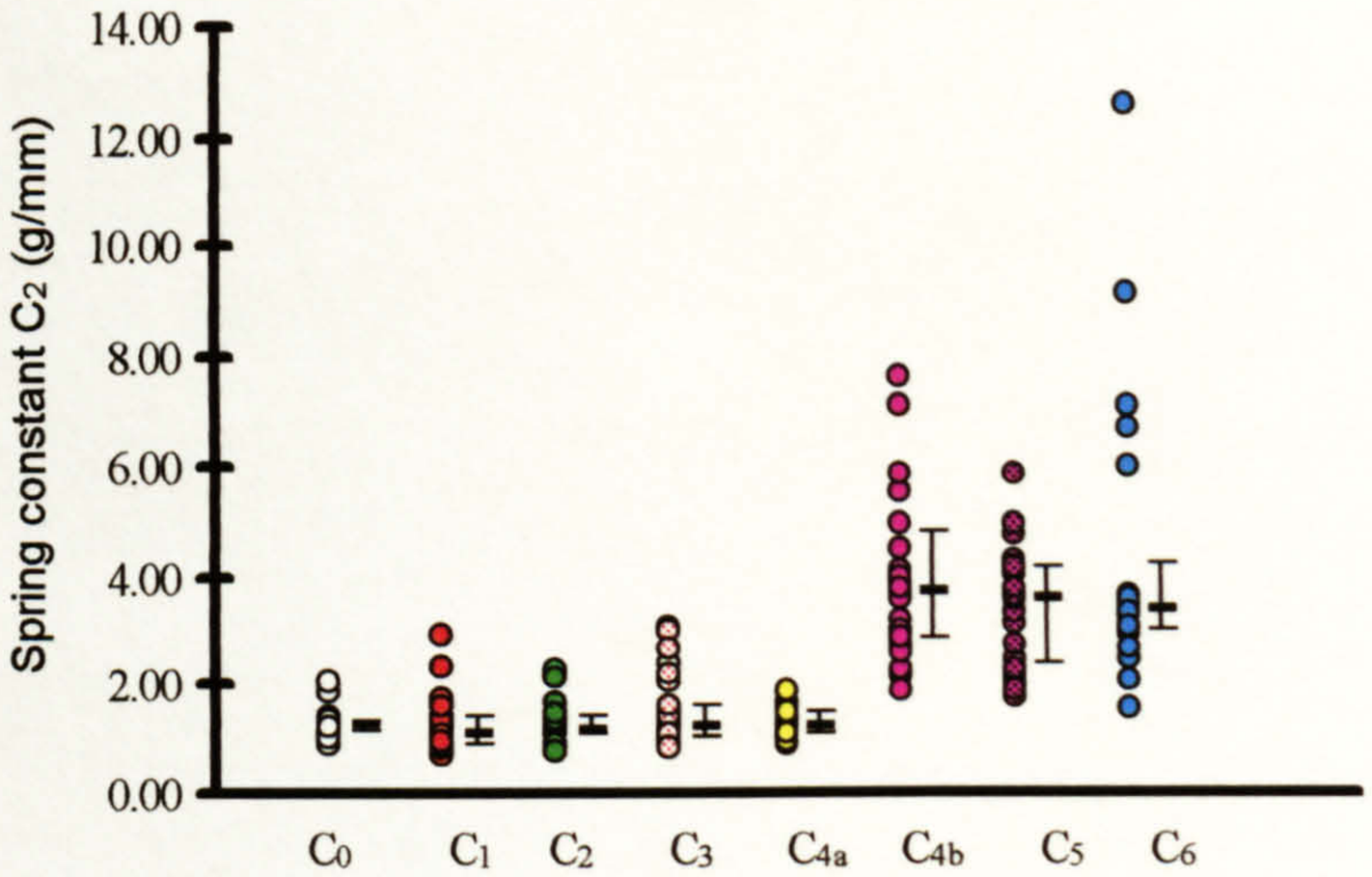
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.62						
C_2	0.05	0.01					
C_3	0.006	0.002	0.32				
C_{4a}	0.0009	0.0002	0.02	0.006			
C_{4b}	0.07	0.04	0.70	0.48	0.05		
C_5	0.82	0.44	0.36	0.32	0.03	0.38	
C_6	0.002	0.0009	0.08	0.14	0.95	0.17	0.04

Figure 3.3.4 Distance travelled by plunger (mm) during phase III in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.18						
C_2	0.59	0.17					
C_3	0.62	0.43	0.67				
C_{4a}	0.38	0.01	0.04	0.03			
C_{4b}	0.35	0.01	0.15	0.12	0.66		
C_5	0.92	0.32	0.87	1.00	0.19	0.19	
C_6	0.78	0.06	0.28	0.36	0.57	0.82	0.49

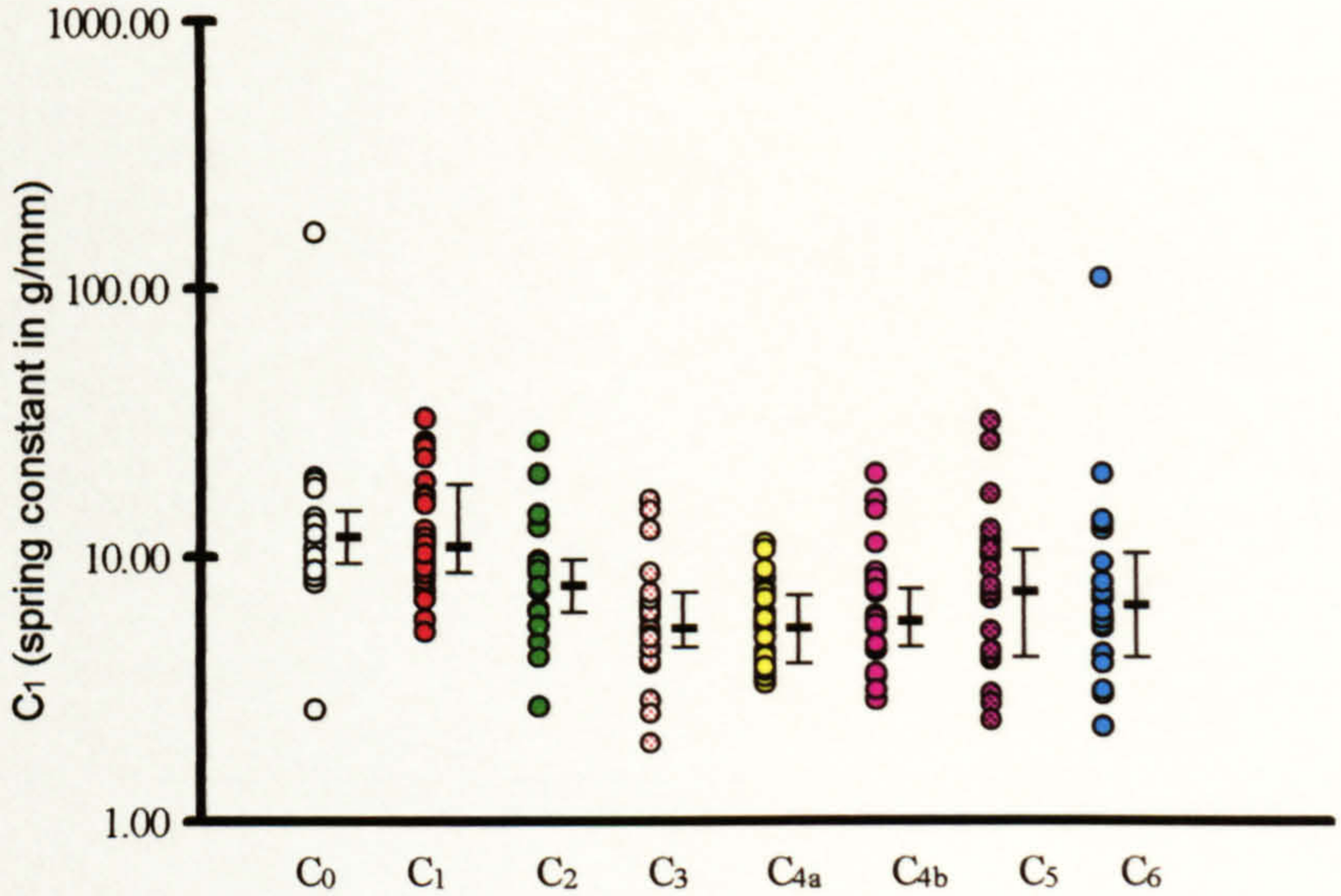
Figure 3.3.5 Rate constant parameter (secs $^{-1}$) during phase III in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.42						
C_2	0.78	0.62					
C_3	0.83	0.41	0.72				
C_{4a}	0.97	0.33	0.38	0.86			
C_{4b}	0.0004	0.0002	0.0002	0.0001	0.0001		
C_5	0.0004	0.0002	0.0002	0.0001	0.0001	0.70	
C_6	0.0004	0.0002	0.0002	0.0001	0.0001	0.96	0.82

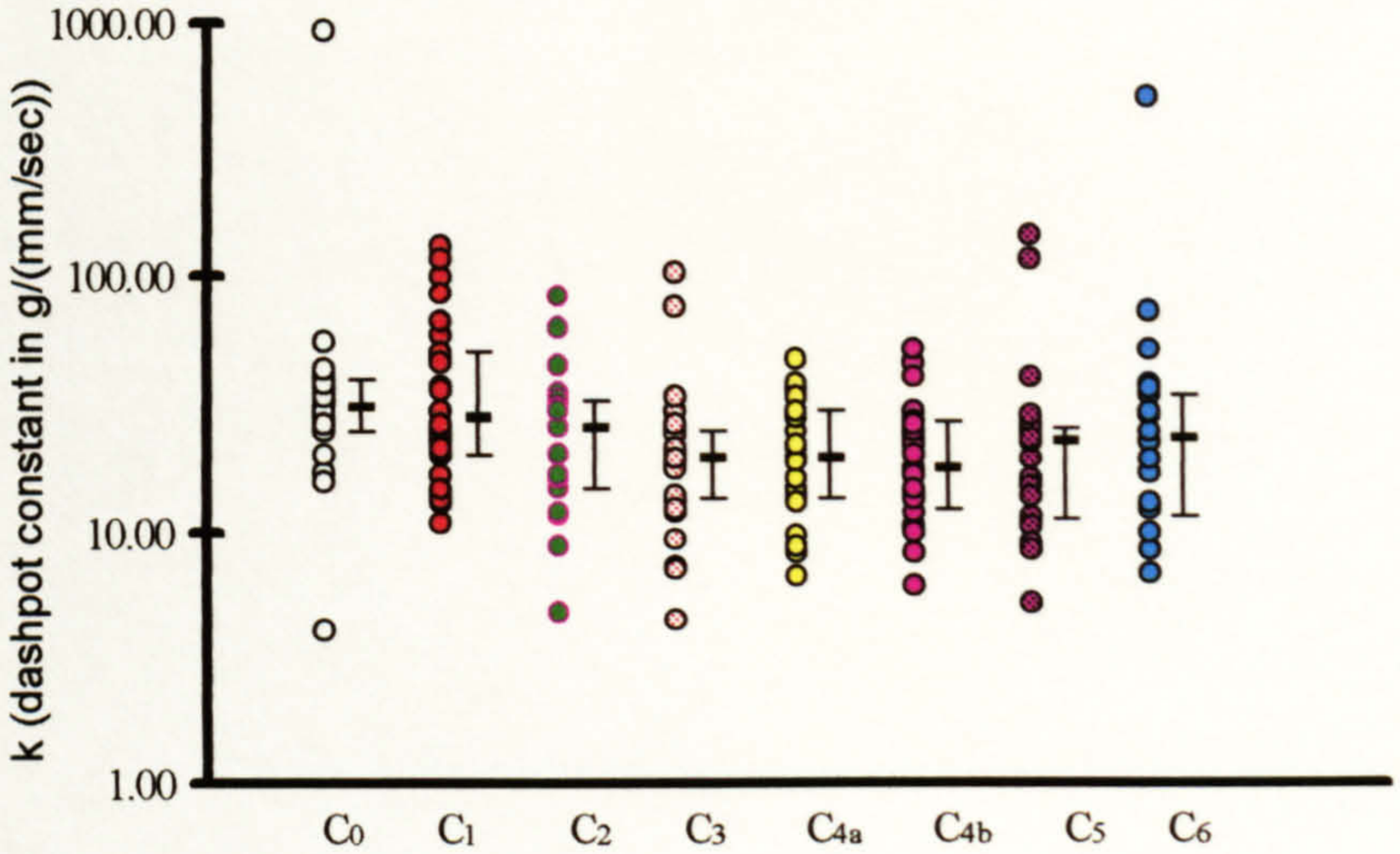
Figure 3.3.6 Series Hooke element, C_2, in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.

Phase II Series Kelvin element, C_1 , vs CEAP classification



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.47						
C_2	0.002	0.05					
C_3	0.006	0.03	0.64				
C_{4a}	0.0007	0.004	0.21	0.11			
C_{4b}	0.0004	0.001	0.04	0.03	0.26		
C_5	0.01	0.09	0.93	0.91	0.38	0.17	
C_6	0.007	0.04	0.54	0.69	0.66	0.32	0.65

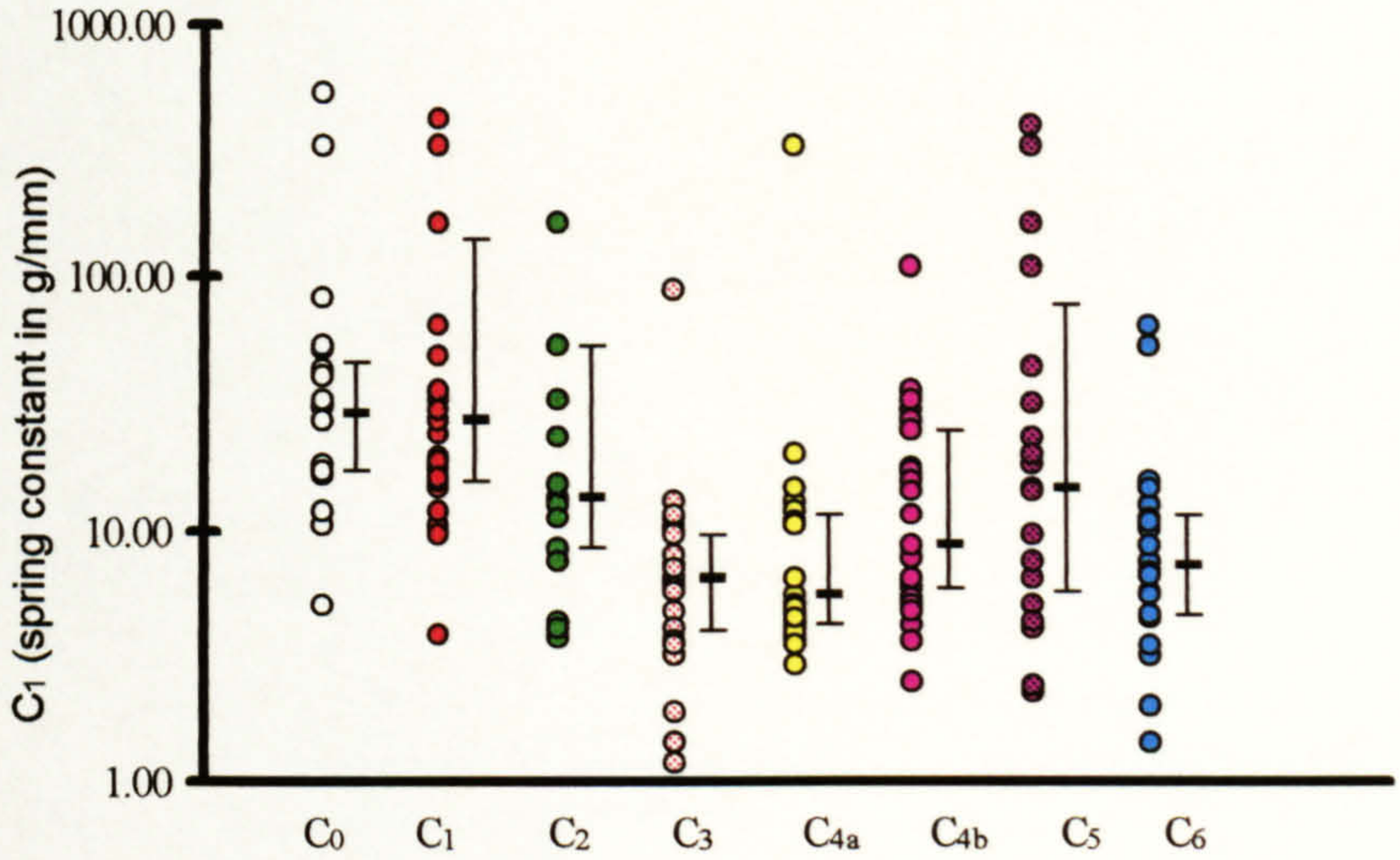
Figure 3.3.7 Series Kelvin element, C_1, during phase II in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.59						
C_2	0.21	0.19					
C_3	0.01	0.43	0.83				
C_{4a}	0.05	0.17	1.00	0.62			
C_{4b}	0.001	0.03	0.44	0.02	0.35		
C_5	0.01	0.22	0.65	0.57	0.90	0.54	
C_6	0.07	0.15	0.82	0.52	0.80	0.36	0.70

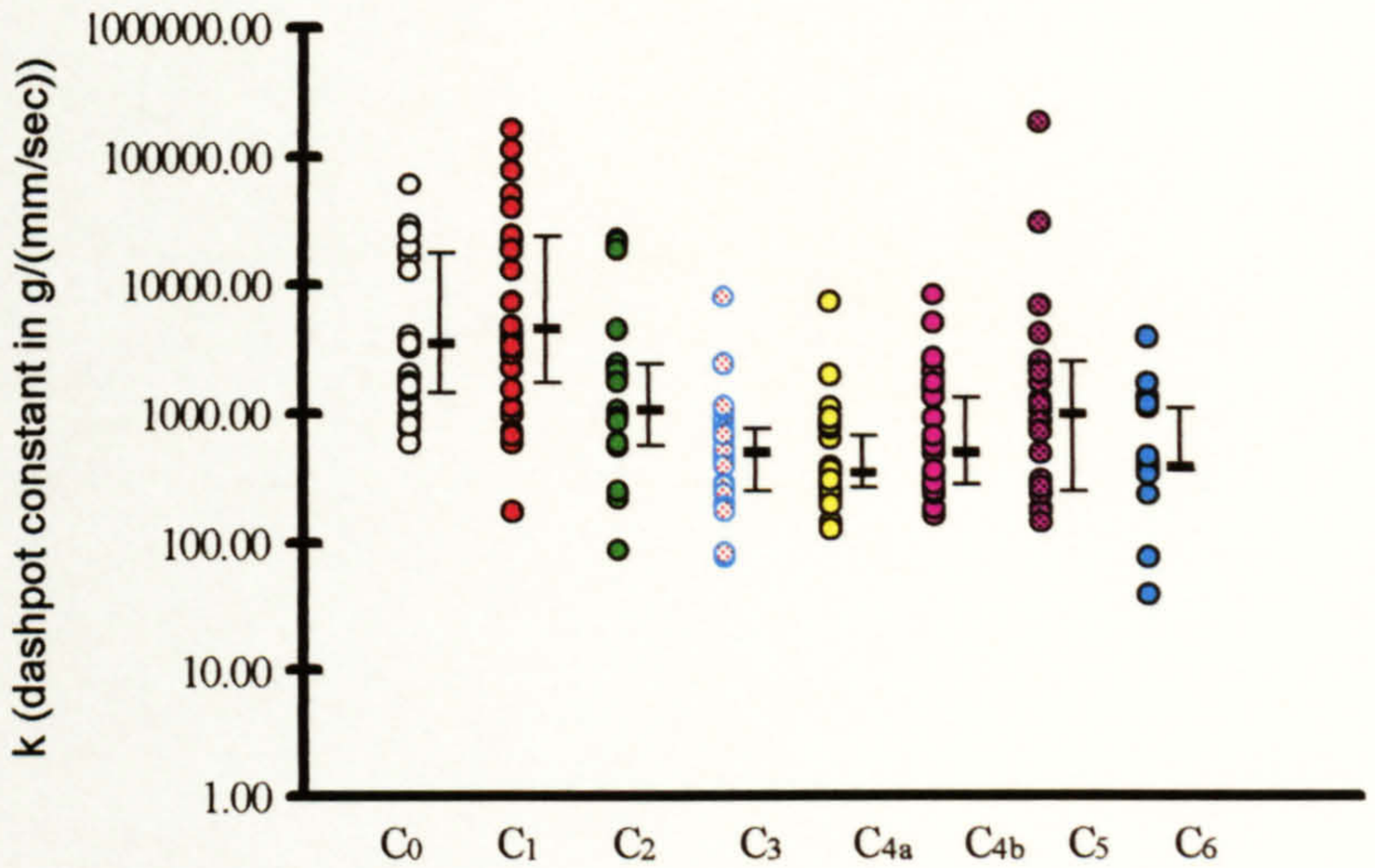
Figure 3.3.8 Dashpot constant, k, during phase II in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.

Phase III Series Kelvin element, C_1 , vs CEAP classification



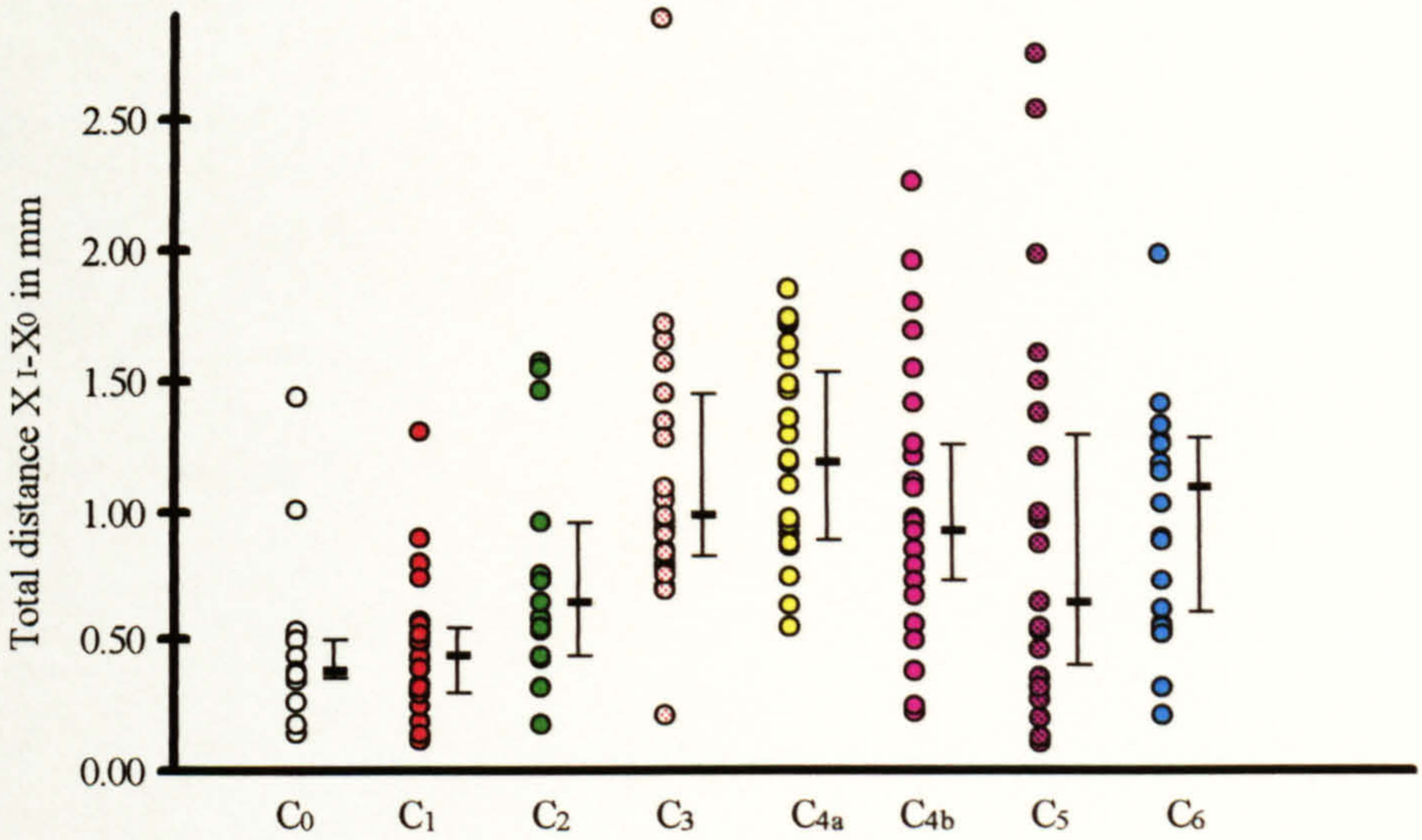
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.59						
C_2	0.05	0.01					
C_3	0.006	0.002	0.32				
C_{4a}	0.0009	0.0002	0.02	0.007			
C_{4b}	0.06	0.03	0.70	0.48	0.05		
C_5	0.85	0.44	0.36	0.32	0.03	0.36	
C_6	0.002	0.0009	0.08	0.15	0.95	0.17	0.04

Figure 3.3.9 Series Kelvin element, C_1, during phase III in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.37						
C_2	0.13	0.02					
C_3	0.06	0.01	0.94				
C_{4a}	0.0009	0.0002	0.03	0.005			
C_{4b}	0.05	0.008	0.54	0.57	0.26		
C_5	0.92	0.40	0.36	0.25	0.02	0.15	
C_6	0.003	0.0009	0.06	0.02	0.45	0.28	0.04

Figure 3.3.10 Dashpot constant, k, during phase III in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



Total distance (X_I-X_0 in mm) travelled by the plunger during phases I and II

CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.89						
C_2	0.005	0.007					
C_3	<0.0005	<0.0005	0.003				
C_{4a}	<0.0005	<0.0005	0.001	0.56			
C_{4b}	0.0001	<0.0005	0.06	0.28	0.13		
C_5	0.03	0.01	0.78	0.06	0.04	0.30	
C_6	0.0001	<0.0005	0.07	0.53	0.27	0.67	0.19

Figure 3.3.11 Distance travelled by plunger (mm) during phases II and III in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.

The distance travelled X_0 by the plunger during phase I, was significantly reduced in the C_{4b}, C_5 and C_6 groups to as much as one third, of that in C_0, C_1, C_2, C_3 and C_{4a} groups (figure 3.3.1).

There were no statistically significant differences in the skin compliance between the C_0, C_1, C_2, C_3 and C_{4a} groups and between the C_{4b}, C_5 and C_6 groups.

The distance travelled X_I-X_0 by the plunger during phase II was significantly higher in the C_2, C_3, C_{4a}, C_{4b}, C_5 and C_6 as compared to the C_0 group. There was no significant difference between the C_0 and C_1 groups and between the C_2, C_3, C_{4a}, C_{4b}, C_5 and C_6 groups (figure 3.3.2).

There were no significant difference in the rate constant parameters during phase II between any of the groups (figure 3.3.3) with a wide scatter in the data points of this parameter amongst the groups.

The distance travelled by the plunger during phase III showed a significant increase between the groups with no significant clinical evidence of oedema (C_0, C_1 and C_2) and the groups with significant clinical evidence of oedema present (C_3, C_{4a} and C_6) (figure 3.3.4). Interestingly, there were no statistically significant difference between the C_0, C_1 and C_2 groups and the C_{4b} and C_5 groups.

There were no significant difference in the rate constant parameters during phase III between any of the groups (figure 3.3.5) with a wide scatter in the data points of this parameter amongst the groups.

C_2, the elastic spring constant of the Hooke element, is significantly increased in the C_{4b}, C_5 and C_6 groups as compared to the C_0, C_1, C_2, C_3 and C_{4a} groups. There were no significant differences in the C_2 parameter between the C_0, C_1, C_2, C_3 and C_{4a} groups and between the C_{4b}, C_5 and C_6 groups (figure 3.3.6).

There was a significant decrease in the C_1 parameter during phase II between the C_0 and C_1 groups and the C_2, C_3, C_{4a}, C_{4b}, C_5 and C_6 groups (figure 3.3.7).

There were no significant difference in the dashpot constant parameter, k, during phase II between the normal controls and the patient groups (figure 3.3.8).

There was a significant difference in the C_1 and k parameters during phase III between the groups with no clinical evidence of tissue oedema (C_0, C_1 and C_2) and the groups with clinical evidence of tissue oedema (C_3, C_{4a}, C_{4b} and C_6) with the exception of the C_5 group (figure 3.3.9 and figure 3.3.10).

Again, there was a significant increase in the total distance travelled by the plunger during the creep phase (X_I-X_0 for phases II and III) between the C_0 and C_1 groups and

the C_2, C_3, C_{4a}, C_{4b} and C_6 groups (figure 3.3.11). However, the C_5 group showed a similar trend as with the other parameters as having a lesser significant difference with the C_0 and C_1 groups.

3.3.5. Discussion

This study was conducted in a much larger range of patients than was included in study I, in order to assess whether the conclusions drawn from the initial series were valid when applied to a further, larger group of subjects. Patients and controls with all severities of venous disease recognised by the clinical part of the CEAP method of classification (C_0 to C_6). Separate groups of patients were included in my C_{4a} and C_{4b} groups, in the same manner as for the earlier study.

The distance travelled X_0 by the plunger during phase I, the initial fast indentation phase, which reflects the skin compliance, was significantly reduced in the C_{4b}, C_5 and C_6 groups to as much as one third, of that in C_0, C_1, C_2, C_3 and C_{4a} groups (figure 3.3.1), a highly statistically significant difference ($P<0.0005$). This was almost identical to my earlier observations on the more limited groups of patients and confirms that they were representative of the patitient groups. The data from this study demonstrates that patients in the C_{4b}, C_5 and C_6 groups had almost identical values for this parameter, confirming my justification for identifying patients with LDS separately and assigning them to group C_{4b}. In addition, the graphs show very little overlap in this measurement between the patients with LDS and those without, confirming its potential for use as a surrogate endpoint for the evaluation of treatments for venous disease.

The distance travelled X_I-X_0 by the plunger during phase II, the subsequent slower indentation phase, probably reflects the amount of blood present beneath the plunger from the considerations presented in section 3.1. Patients with any severity of venous disease of C_2 (uncomplicated varicose veins) or greater had an increase in this parameter. This is consistent with my earlier proposal that this measurement reflects an increase in the venous volume due to dilated veins (small and large) in the skin and subcutaneous tissues. A number of plethysmographic studies have also shown increased venous volume in patients with venous disease compared to controls, so this observation is not surprising. In the C_5 and C_6 groups, this parameter decreased compared with the measurement in patients with less severe venous disease. This again suggests the likelihood that the travel of the plunger was limited by the reduced tissue compliance in the patients with severe LDS.

There were no significant difference in the rate constant parameters during phase II between any of the groups (figure 3.3.3) with a wide scatter in the data points of this parameter amongst the groups. The wide scatter of these data and overlap in both the distance and rate constant parameters makes it less likely that this could be used as an indicator of the severity of venous disease.

The distance travelled by the plunger during phase III showed a significant increase between the groups with no significant clinical evidence of oedema (C_0, C_1 and C_2) and the groups with significant clinical evidence of oedema present (C_3, C_{4a} and C_6) (Figure 3.3.4). Interestingly, there were no statistically significant difference between the C_0, C_1 and C_2 groups and the C_{4b} and C_5 groups. Again, this probably reflects reduced tissue compliance preventing accurate assessment of oedema.

There were no significant difference in the rate constant parameters during phase III between any of the groups (figure 3.3.5) with a wide scatter in the data points of this parameter amongst the groups. This parameter is of use in assessing the severity of oedema, but has the limitations that the reproducibility studies showed it to have a large coefficient of variation on repeated measurement. In addition, the interaction with reduced tissue compliance in patients with LDS means that it would not be useful in these patients. The considerable overlap between patient groups means that it would be of limited value in distinguishing different severities of venous disease.

C_2, the elastic spring constant of the Hooke element which is a measure of the skin compliance (the higher the constant the lower the compliance), is significantly increased in the C_{4b}, C_5 and C_6 groups as compared to the C_0, C_1, C_2, C_3 and C_{4a} groups (figure 3.3.6) (remember that C_2 is inversely related to the X_0 parameter calculated in figure 3.3.1). There were no significant differences in the C_2 parameter between the C_0, C_1, C_2, C_3 and C_{4a} groups and between the C_{4b}, C_5 and C_6 groups.

According to the Kelvin-Hooke model, the C_1 parameter which is the spring constant of the Kelvin element, is inversely related to the final distance travelled (X_1-X_0) by the plunger. In other words the value of C_1 should be lower in oedematous limbs. There was a significant decrease in this parameter during phase II between the C_0 and C_1 groups with no clinical evidence of tissue oedema, and the C_2, C_3, C_{4a}, C_{4b}, C_5 and C_6 groups which have clinical evidence of tissue oedema (figure 3.3.7). This parameter may be useful in indicating the amount of expansion of venous volume especially during the earlier stages of the creep phase which occurs within the first 10 seconds (phase II).

There were no significant difference in the dashpot constant parameter, k, during phase II between the normal controls and the patient groups (figure 3.3.8).

There was a significant difference in the C_1 and k parameters during phase III between the groups with no clinical evidence of tissue oedema (C_0, C_1 and C_2) and the groups with clinical evidence of tissue oedema (C_3, C_{4a}, C_{4b} and C_6) with the exception of the C_5 group (figure 3.3.9 and figure 3.3.10). According to the Kelvin-Hooke model, as the distance travelled by the plunger (X_1-X_0) increased due to the increase in tissue fluids present beneath the plunger, the value of the C_1 and k parameters will decrease. This is expected as the C_1 and k values are inversely related to the X_1-X_0 values. Interestingly, the C_5 group had a higher C_1 and k values than the other groups with clinical evidence of tissue oedema present possibly reflecting a wider spread of patients with varying degrees of the extent of the liposclerotic changes as can be seen from the X_0 parameter in figure 3.3.1.

3.3.6. Conclusion

This study has shown that the earlier observations are correct when applied to a new, larger group of patients. This investigation confirms that the skin compliance in patients with venous disease can be reliably assessed by the tonometer. It also continues to justify the separation of patients into C4a and C4b groups. The latter group (LDS) is clearly very similar to the patients with open or healed venous ulcers. The Kelvin-Hooke model appeared to fit the data quite well in assessing the mechanical properties of the skin of patients with lower limb chronic venous disease.

3.4 STUDY IV

To correlate the clinical assessment of the hardness of liposclerotic skin changes with tissue tonometry measurements of skin compliance.

3.4.1. Rationale

Doctors generally demonstrate the principles of tonometry by palpating skin and subcutaneous tissues with their fingers to see how soft or hard it is by the extent with which their fingers will sink into the tissue. However, this method of assessment of the skin compliance is subjective and qualitative. Similarly, the clinical assessment of skin compliance and oedema is also subjective and qualitative. There is no objective measurement of the ease of indenting the skin surface or the ease with which fluid moves away from compression of the tissues with the thumb as the pressure applied by the thumb is arbitrary. Therefore changes in the skin compliance or the extent and rate of pitting cannot be quantitatively assessed.

The severity of the induration or skin hardness has never been objectively quantified. The clinical assessment of lipodermatosclerosis at present uses the clinical skin severity score index adapted from studies of patients with systemic sclerosis (scleroderma) adapted by Kahaleh et al in 1986. This is very subjective and clinical assessment will vary from laboratory to laboratory.

Recently a durometer has been used to determine the degree of induration in LDS by Romanelli (Romanelli et al, 1995) based on previous work done on the assessment of skin hardness in patients with systemic sclerosis (scleroderma) by Falanga (Falanga et al, 1993).

I have previously objectively assessed the skin in patients with lower limb chronic venous disease using a tissue tonometer developed in our laboratory and shown that the skin compliance is reduced to about a third in patients with liposclerotic skin changes and palpable induration (C_{4b}) as compared to patients with liposclerotic skin changes without palpable induration (C_{4a}), patients with varicose veins and clinical evidence of oedema (C_3) and normal controls (C_0).

Results obtained *in vitro* and *in vivo* with the tissue tonometer developed in our laboratory (see study II and III) have been shown to fit a simple visco-elastic mathematical model used to analyse the skin compliance. The X_0 (mm) distance

parameter during the initial rapid indentation phased and the spring constant, C_2, calculated from the Kelvin-Hooke model are used as indicators of the skin compliance.

3.4.2. Aim

To compare the clinical assessment of the severity of liposclerotic skin changes made by an experienced vascular surgeon with the tissue tonometry parameters of skin compliance (X_0 (mm) and the C_2 spring constant (g/mm)) in patients with bilateral LDS.

3.4.3. Methodology

Thirty two limbs of 16 patients (9M:7F; mean age of 64.1 years; range 43-78 years) referred to the vascular laboratory with bilateral lipodermatosclerosis (modified CEAP class C_{4b}) were assessed clinically by an experienced specialist registrar in vascular surgery followed by tissue tonometry measurements performed by the author. They all had a full non-invasive venous duplex ultrasound examination to confirm presence of lower limb venous disease. Resting ABPI were measured to eliminate any arterial disease. The clinician used the CEAP method of scoring the liposclerotic skin changes as 1 point for less severe CEAP class C_{4b} and 2 points for more severe CEAP class C_{4b}. Tissue tonometry measurements were carried out on the medial aspect of the gaiter area above the medial malleolus on all 32 limbs as described in section 2.8. Three sites on each limb (6cm, 10cm and 12cm above the medial malleolus) were measured to get a mean value. The results of scoring of the liposclerotic skin changes by the clinician remained unknown to the author who performed the tissue tonometry measurements.

Data Analysis

The tonometry parameters of X_0 in mm and the spring constant C_2 were plotted against the clinical severity scores of 1 and 2 points for less severe LDS and more severe LDS respectively, as scored by the clinician. The median and inter-quartile ranges were calculated and the Wilcoxon test was applied to test for statistical significance.

3.4.4. Results

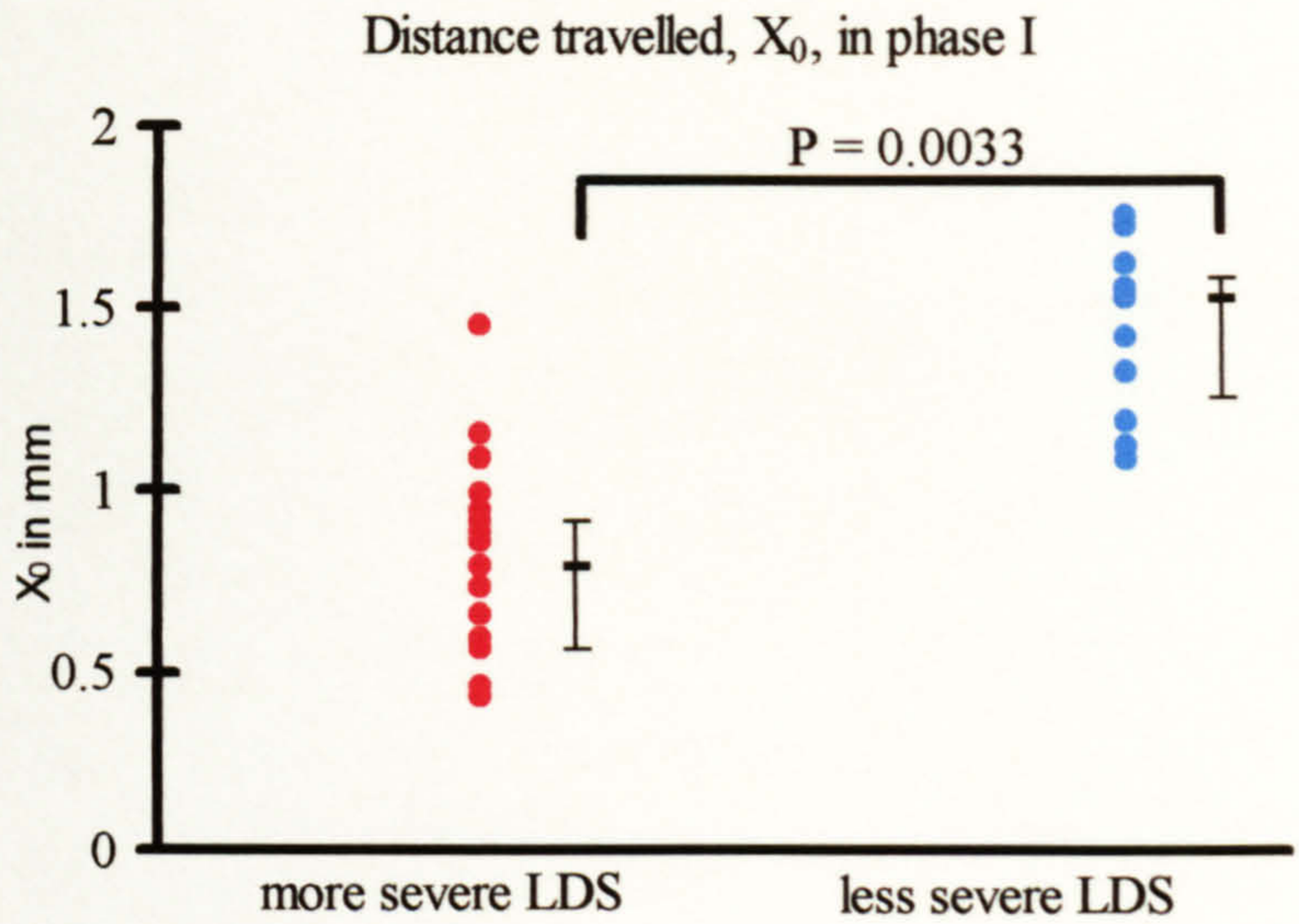


Figure 3.4.1 The distance travelled by the plunger in the first second is plotted against the clinical severity of the LDS as classified by a vascular surgeon.

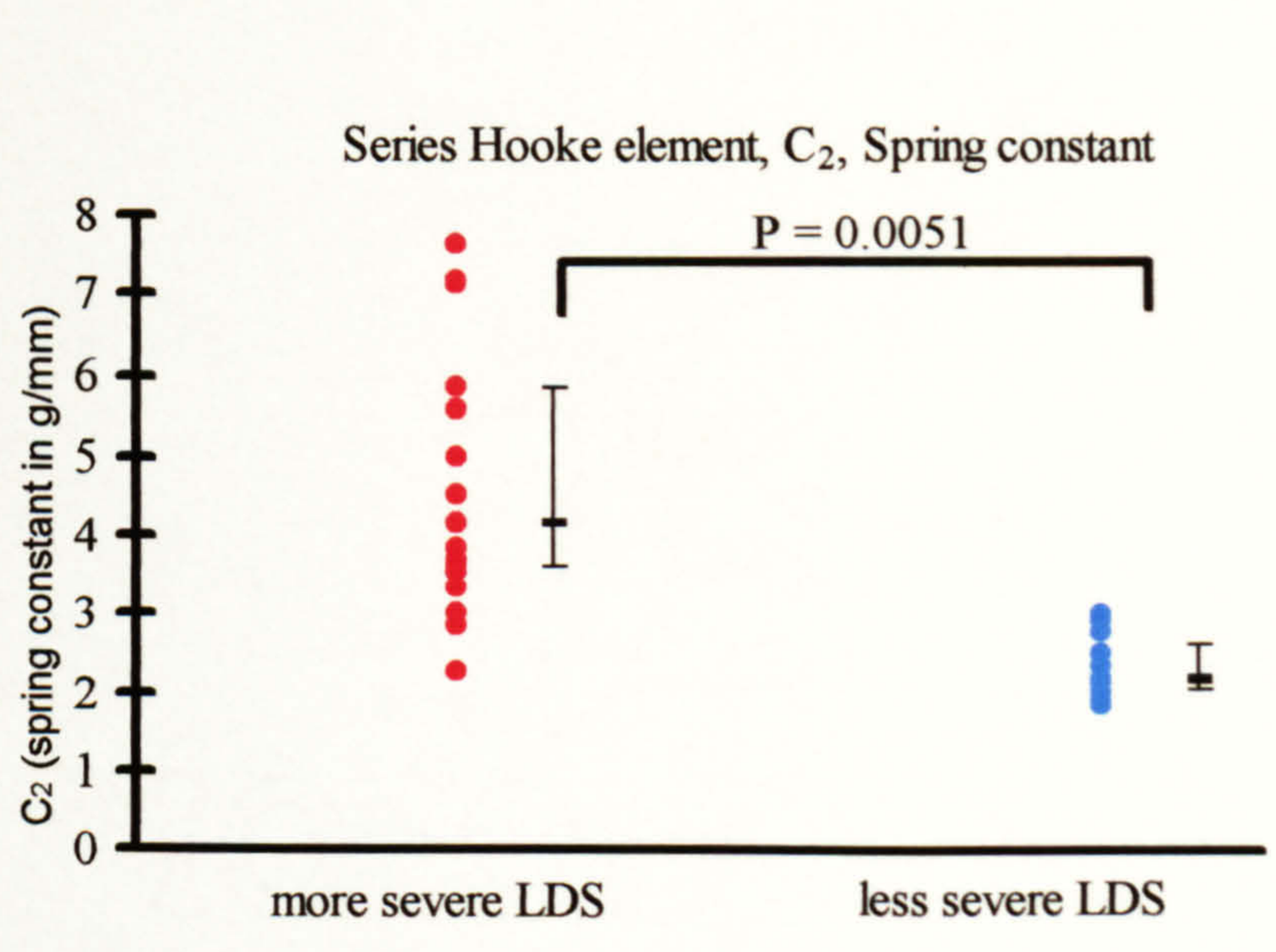


Figure 3.4.2 The spring constant, C_2, calculated from the Kelvin-Hooke model is plotted against the clinical severity of the LDS as classified by a vascular surgeon.

Differentiating between the clinical severity of skin changes within the CEAP class C_{4b} was similar between the X_0 and C_2 parameters obtained by tissue tonometry and the clinical severity as classified clinically by a vascular surgeon and this difference reached statistical significance (figures 3.4.1 and 3.4.2) ($p=0.0033$ for the X_0 parameter and $p=0.0051$ for the C_2 parameter). There was some overlap in the tissue tonometry parameters of skin compliance between the less severe and more severe liposclerotic skin changes.

3.4.5. Discussion

The aim of this investigation was to determine whether the severity of LDS suggested by a clinician correlated with that measured by the tonometer. In fact, there was very little overlap between the two groups when assessed by the X_o parameter in phase I. This is a remarkable achievement for both the clinician and the measuring technique and confirms that this measurement reliably assess the severity of LDS. The other phases of the tonometer trace showed differences between clinical CEAP stages but did not appear to reliably reflect the severity of lipodermatosclerosis, the most important clinical indicator of the severity of venous disease. Consideration of these was therefore omitted in this study. In this study, I was only interested in the C_2 elastic spring constant of the Hooke element, which represents the initial ability of the interstitial matrix to undergo elastic deformation without flow. The other parameter of interest was the X_0 parameter which is the distance travelled by the plunger during phase I, the initial fast indentation phase. Both these parameters reflect the skin compliance and are inversely related. The X_0 parameter is calculated from the straightest part of the tonometer curve which occurs within 1 second as the plunger was released unto the skin.

The skin compliance parameters as measured by tissue tonometry has been previously shown to be very reproducible in patients (COV=3.8%) and in normal controls (COV = 8%) and has also proved to be quite a sensitive method of investigation in this study. It has the advantage over clinical examination of being very reproducible and entirely objective, being uninfluenced by the other clinical features of the limb.

3.4.6. Conclusion

This study demonstrated that the tissue tonometry measurements of skin compliance provided an objective way of differentiating between the clinical severity of liposclerotic skin changes in patients all of whom were affected by LDS.

3.5 STUDY V

Tissue tonometry measurements of skin compliance and its correlation with ultrasound measurement of thickness of the skin and subcutaneous tissues.

3.5.1. Rationale

The distance travelled by the plunger during the initial fast indentation phase, X_0 in mm, should depend only on the initial elastic compressibility or skin compliance. There was a variable range of values of the X_0 parameter in studies I and III within the normal and patient groups and this may possibly reflect variation in skin thickness or skin and subcutaneous fat thickness.

Current methods of studying the skin includes histology and electron microscopy but the most practical modality is ultrasound imaging.

Ultrasound does not have the resolution of histology but a major advantage of using ultrasound is it provides real-time imaging capability and it is non-invasive.

Ultrasound examination of the skin itself is best performed with 20-MHz transducers but has limitations which include the narrow field of view which is less than 2cm in width and the inability to visualise the subcutaneous tissues beyond a depth of approximately 1.5cm. These limitations emphasise the need for lower frequency transducers in the 7.5 to 10 MHz range to image structures that lie beyond the scope of the 20-MHz transducers and also because of their low cost and wider availability.

The use of 7.5- and10-MHz transducers are ideal for use in the ultrasound imaging of the subcutaneous tissues with the added advantages of being low cost, versatile, quicker to perform, more widely available than any other cross-sectional imaging modality currently available and display a pictorial cross-section of the skin and subcutaneous tissues in a visual way.

3.5.2. Aims

To measure skin and subcutaneous layer thickness in the normal and patient groups presenting with lower limb venous disease using a 7.5 MHz transducer.

To obtain displacement versus time using a tissue tonometer within the same groups above.

To determine the correlation between the distance and rate constant parameters obtained with tissue tonometry and the ultrasound measurements of skin and subcutaneous layer thickness.

3.5.3. Methodology

Thirty seven limbs of twenty nine subjects (13M:16F; mean age of 66 years; range: 47-75 years) confirmed with presence of lower limb venous disease by duplex ultrasound scanning in the vascular laboratory and twelve limbs of twelve normal controls (8M:4F; mean age of 29 years; age range: 21-37 years) with no evidence of lower limb venous or arterial disease were also investigated. The subjects were classified according to the CEAP method of classification: and C_0 (n=12), C_2 (n=7), C_{4a} (n=5) and C_{4b} (n=25).

3.5.3.1. Ultrasound

The subject was examined in the supine position using a 7.5MHz linear-array probe as described in section 2.9.

3.5.3.2. Tissue tonometry measurements of skin compliance and tissue tonicity

The tissue tonometer was positioned on the same site as the ultrasound measurement as described in section 2.8 on the medial aspect of the gaiter area of the lower extremity with the subject in the supine position.

Data Analysis

The traces were analysed for distance parameters as described in section 1.5.2.

The Pearson correlation coefficient values were calculated for the ultrasound measurements of skin and subcutaneous layer and the tissue tonometry measurements of distance parameters (X_0, X_I-X_0 during phase II, and X_I-X_0 during phase III) between the CEAP groups investigated.

3.5.4. Results

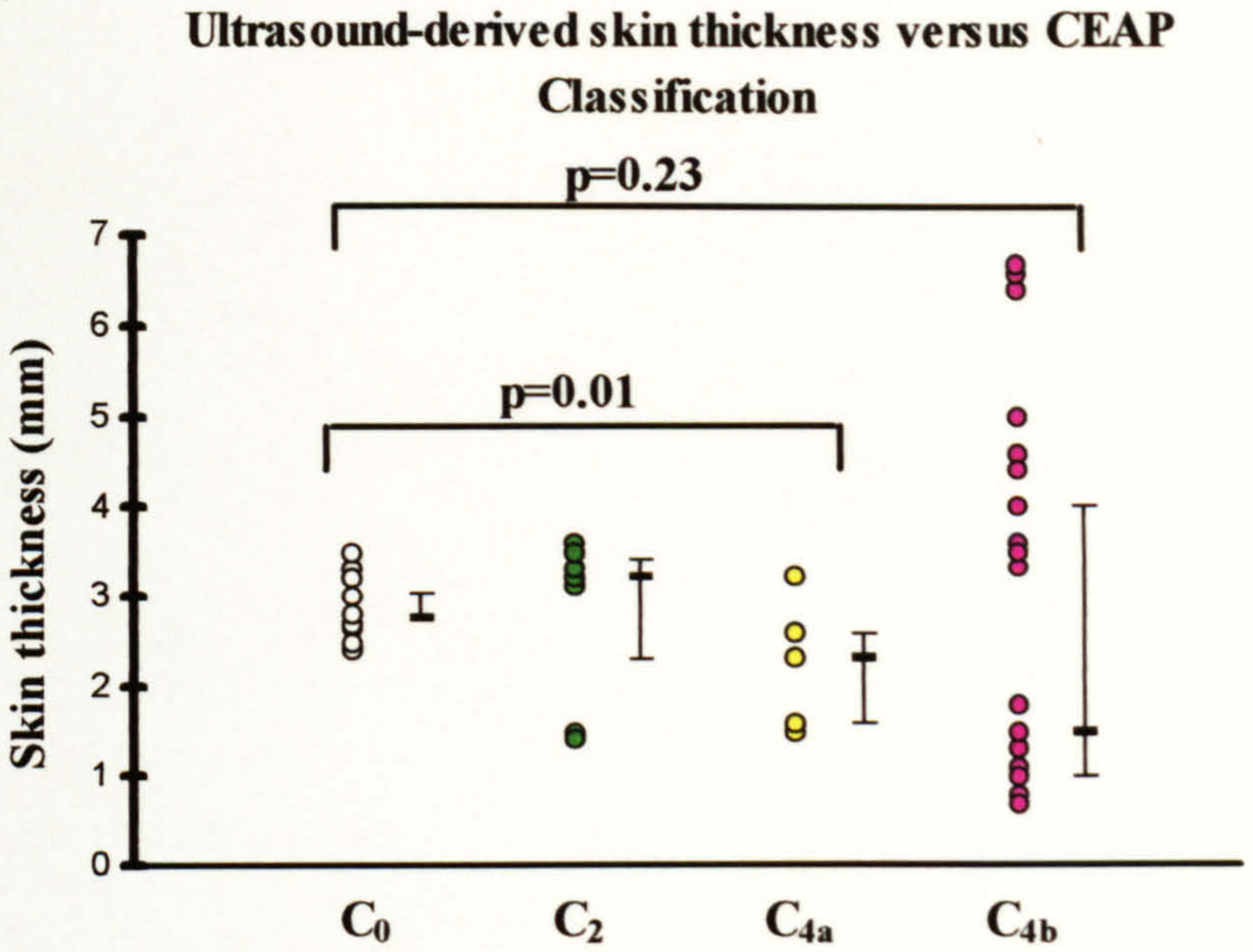


Figure 3.5.1 Ultrasound-derived skin thickness in mm plotted against CEAP classification with the differences between the groups indicated by the p values.

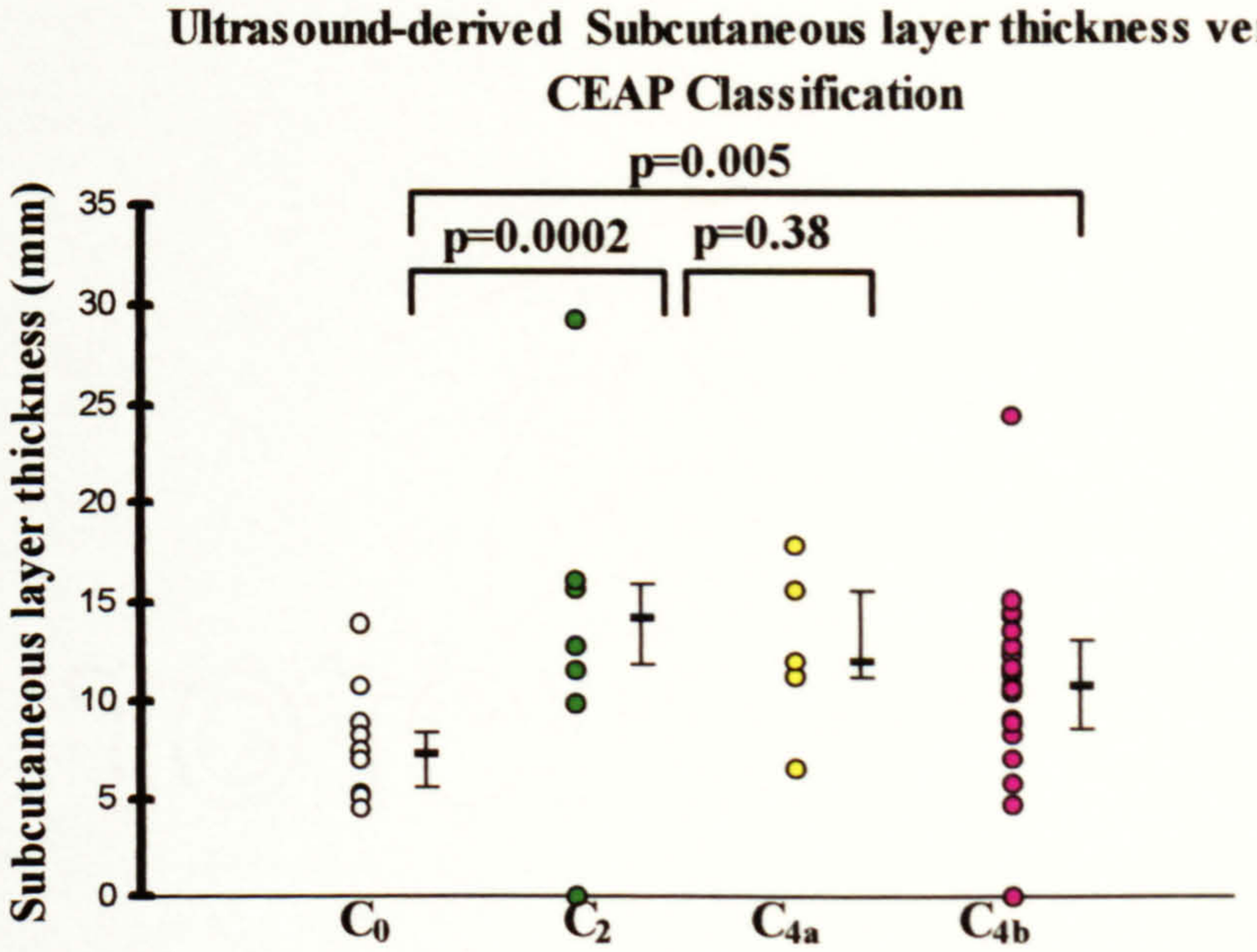


Figure 3.5.2 Ultrasound-derived subcutaneous layer thickness in mm plotted against CEAP classification with the differences between the groups indicated by the p values.

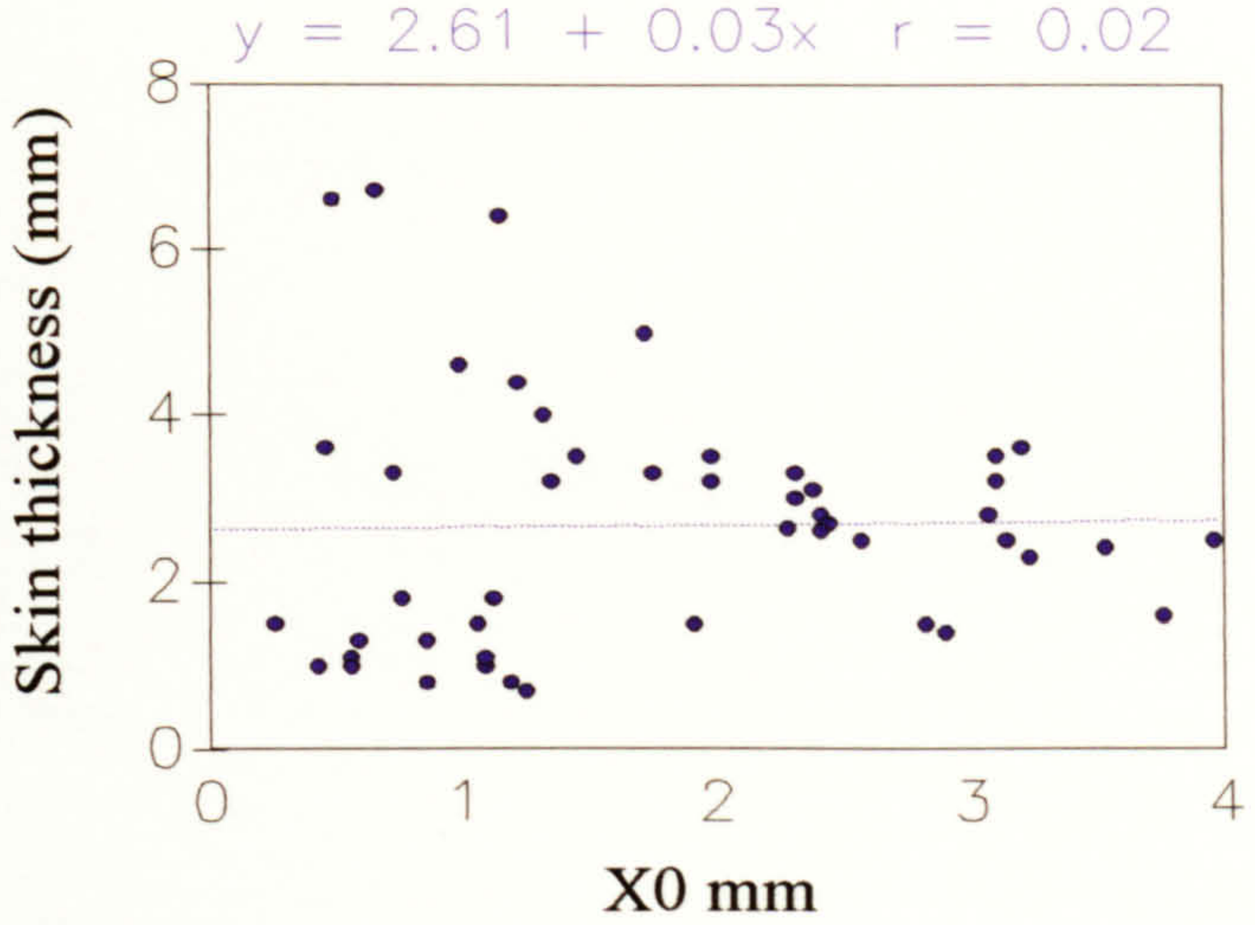


Figure 3.5.3 Regression analysis to show the correlation between ultrasound-derived skin thickness measurement and tissue tonometry measurement of distance travelled during phase I in normal controls and patients with venous disease (C2, C4a, C4b)

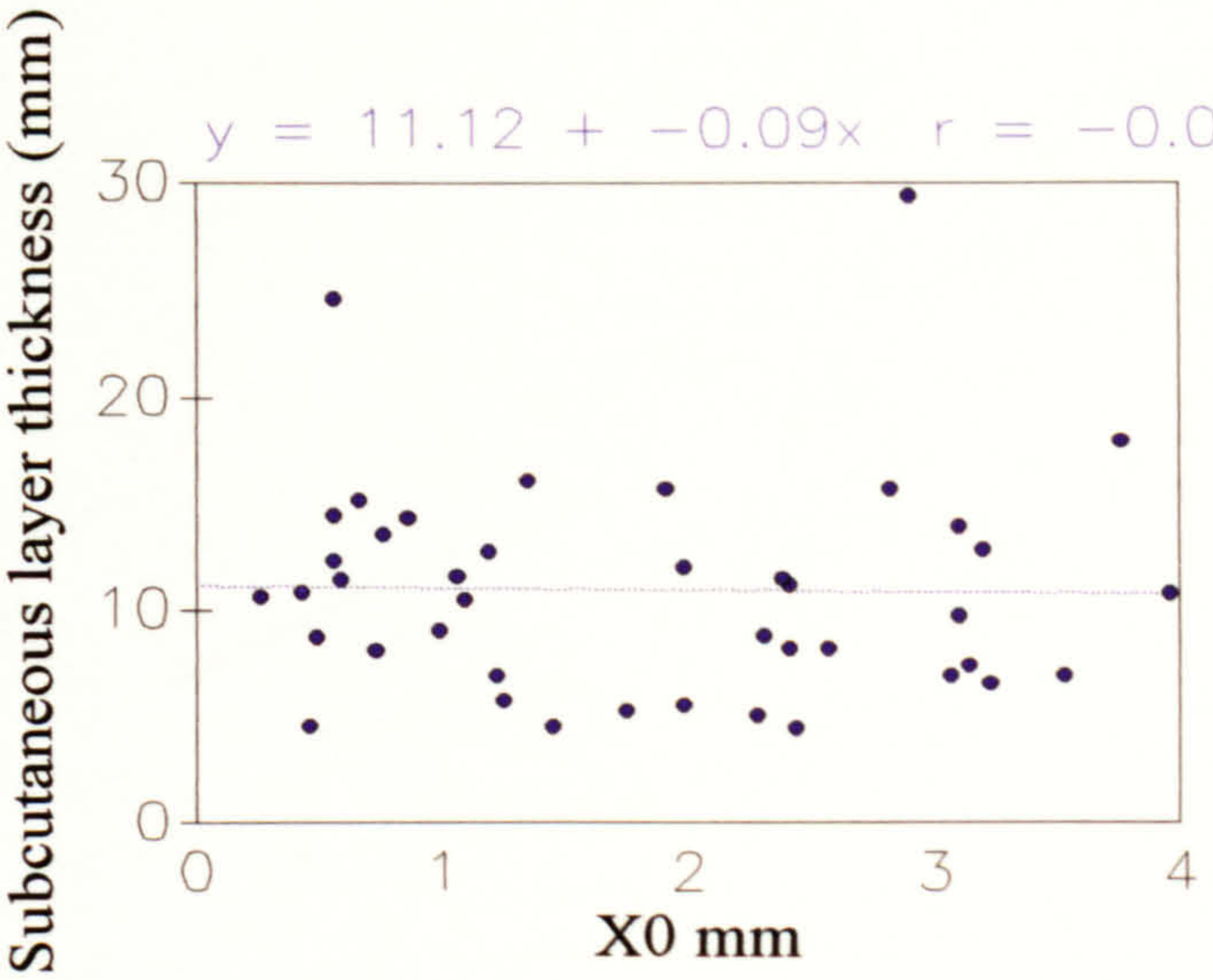


Figure 3.5.4 Regression analysis to show the correlation between ultrasound-derived subcutaneous layer thickness measurement and tissue tonometry measurement of distance travelled during phase I in normal controls and patients with venous disease (C2, C4a, C4b).

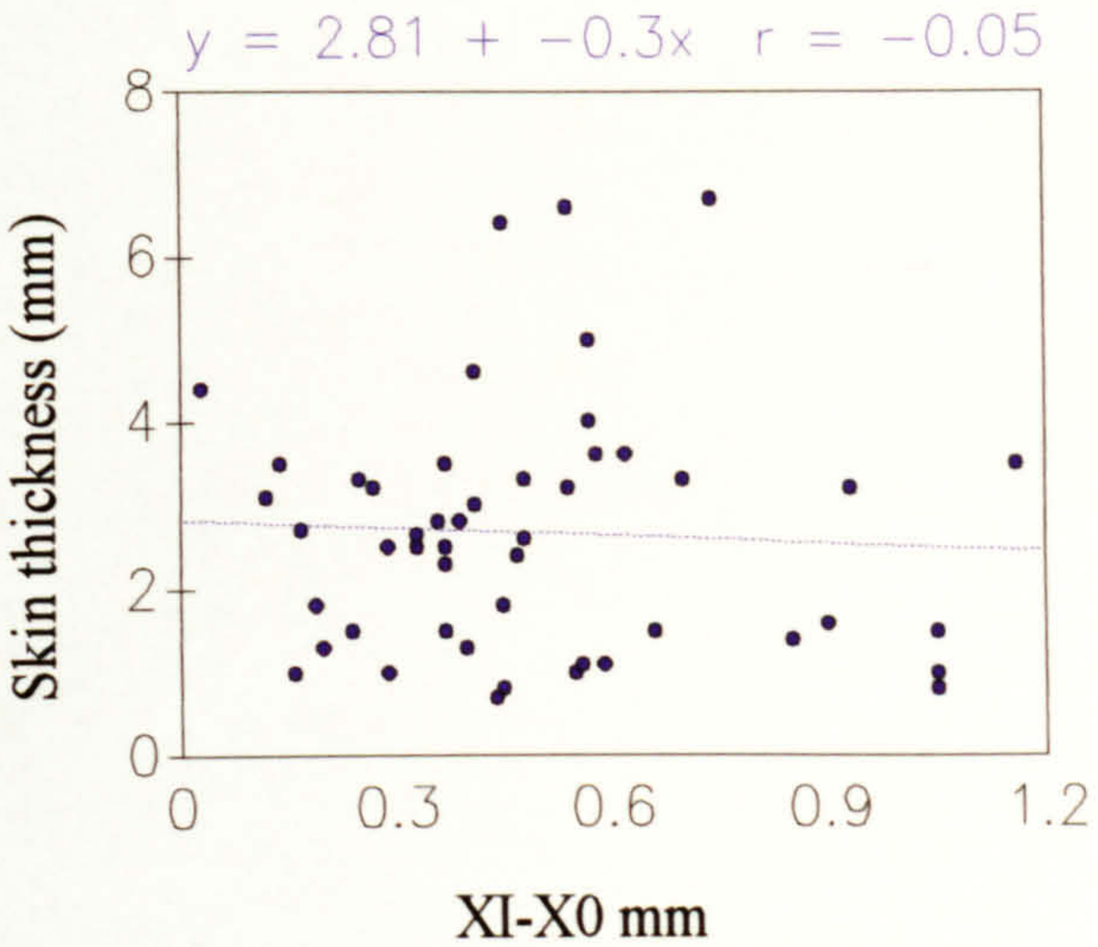


Figure 3.5.5 Regression analysis to show the correlation between ultrasound-derived skin thickness measurement and tissue tonometry measurement of distance travelled during phase II in normal controls and patients with venous disease (C2, C4a, C4b).

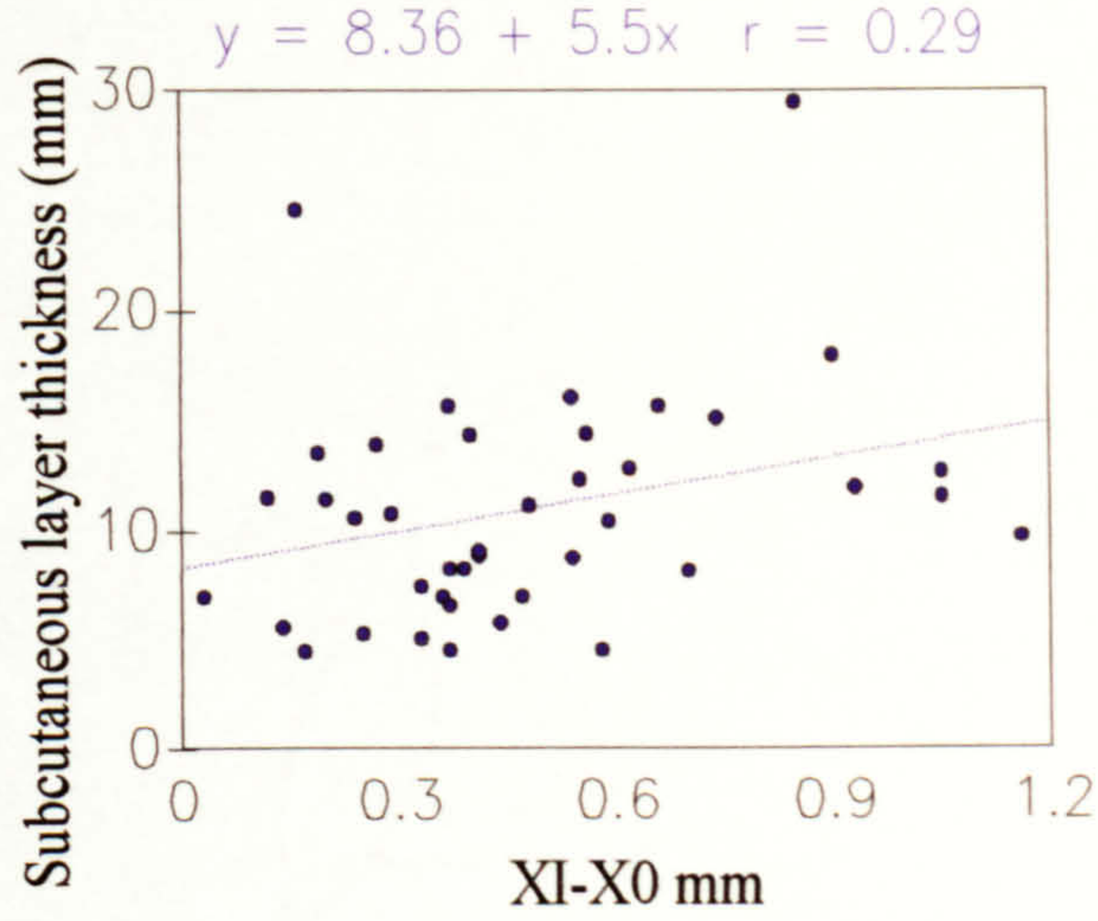


Figure 3.5.6 Regression analysis to show the correlation between ultrasound-derived subcutaneous layer thickness measurement and tissue tonometry measurement of distance travelled during phase II in normal controls and patients with venous disease (C2, C4a, C4b).

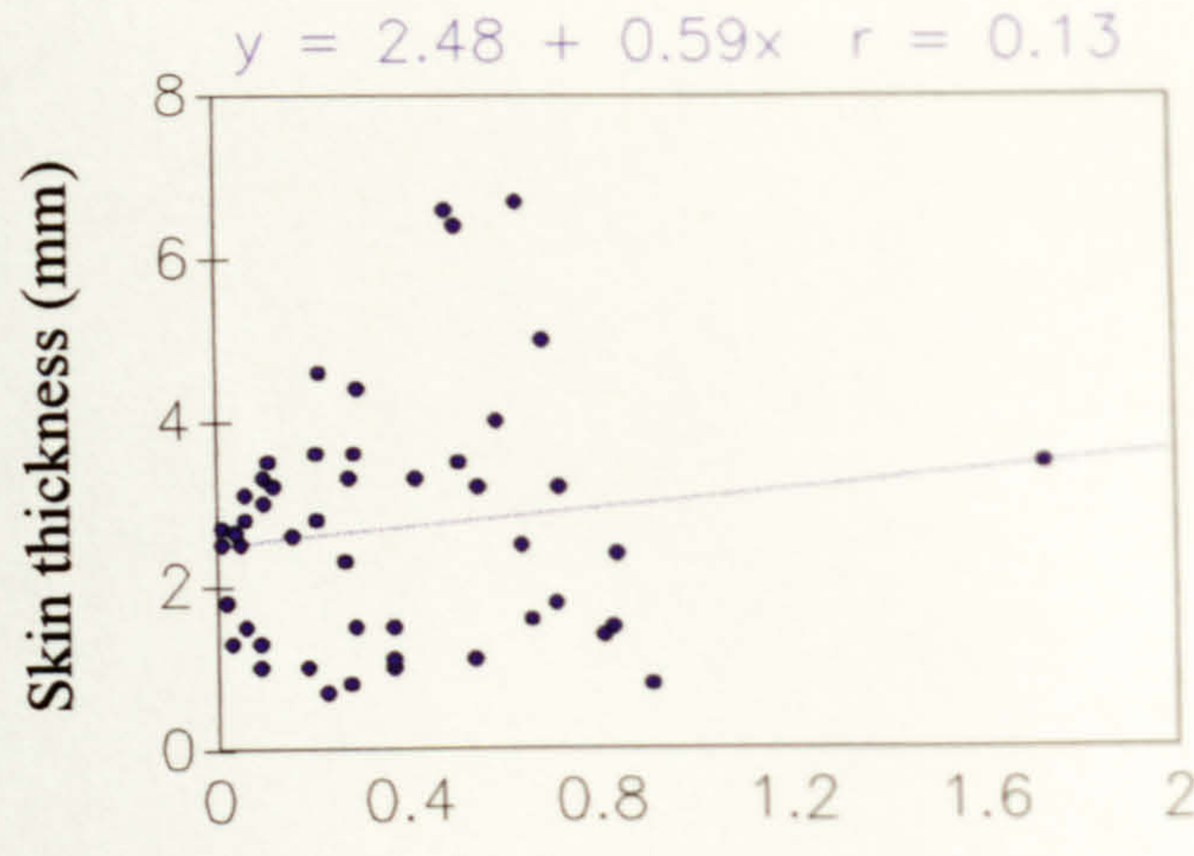


Figure 3.5.7 Regression analysis to show the correlation between ultrasound-derived skin thickness measurement and tissue tonometry measurement of distance travelled during phase III in normal controls and patients with venous disease (C2, C4a, C4b).

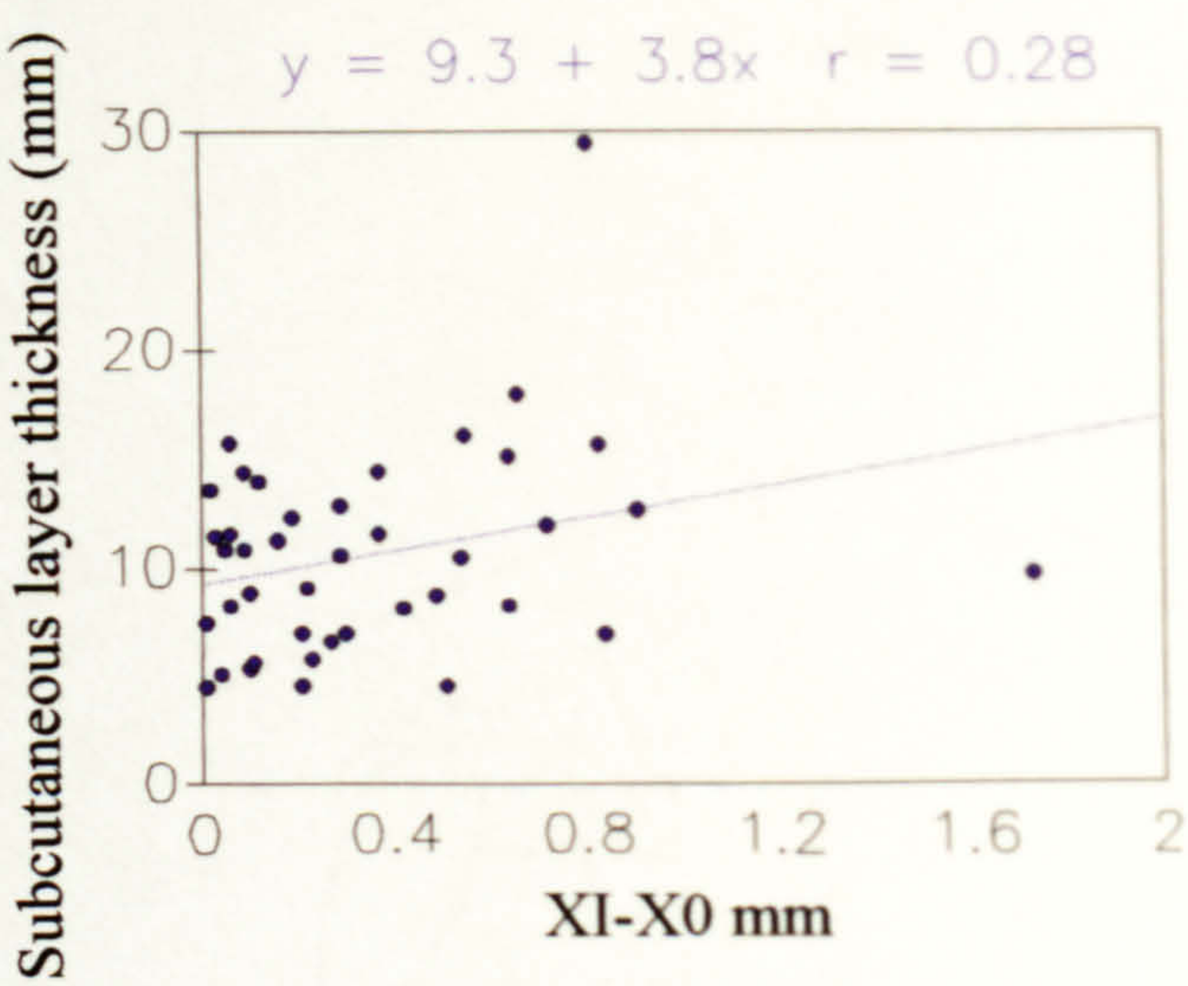


Figure 3.5.8 Regression analysis to show the correlation between ultrasound-derived subcutaneous layer thickness measurement and tissue tonometry measurement of distance travelled during phase III in normal controls and patients with venous disease (C2, C4a, C4b).

Summary of results

Tissue tonometry measurements (C_0,C_2,C_{4a} and C_{4b})	**Pearson correlation value**	
	Ultrasound measurements	
	Skin (mm)	**Subcutaneous Layer (mm)**
X_0 (mm)	0.02	-0.02
X_I-X_0 (mm) phase II	- 0.05	0.29
X_I-X_0 (mm) phase III	0.13	0.28
	Pearson correlation value	
Tissue tonometry measurements (C_0,C_2 and C_{4a})	**Ultrasound measurements**	
	Skin (mm)	**Subcutaneous Layer (mm)**
X_0 (mm)	- 0.31	0.10
X_I-X_0 (mm) phase II	- 0.16	0.53
X_I-X_0 (mm) phase III	- 0.09	0.34
	Pearson correlation value	
Tissue tonometry measurements (C_{4b})	**Ultrasound measurements**	
	Skin (mm)	**Subcutaneous Layer (mm)**
X_0 (mm)	0.15	- 0.32
X_I-X_0 (mm) phase II	- 0.02	- 0.05
X_I-X_0 (mm) phase III	0.33	0.03

Table 3.5.1 Summary of the Pearson correlation values between ultrasound measurements and tissue tonometry.

There was no significant difference in the skin thickness layer between normal controls and patient groups with a wide scatter of data points present (figure 3.5.1).

There was a significant difference in the subcutaneous layer thickness between the C_0 group and the disease groups, C_2, C_{4a} and C_{4b} (figure 3.5.2).

There was no significant correlation between the ultrasound-derived skin and subcutaneous layer thickness and the X_0 and X_I-X_0 parameters during phases II and III obtained from tissue tonometry (see figures 3.5.3 to 3.5.8 and table 3.5.1).

3.5.5. Discussion

Ultrasound is widely used to evaluate changes in the thickness of subcutaneous fat associated with various clinical conditions in humans (Gooding et al, 1986; Heckmatt et al, 1988). Ultrasound has also been used to measure the thickness of subcutaneous fat at various sites in normal subjects (Maruyama et al, 1991) using a thin stand-off pad to

improve the examination of the skin and superficial subcutaneous tissues which results in the skin being closer to the focal zone (Fornage et al, 1984).

Previous studies done using a 7.5MHz linear-array probe, described the skin's appearance as a thin, regular layer of tissue that is more echogenic and sharply demarcated from the underlying hypoechoic subcutaneous fat (Fornage et al, 1986; Miyauchi et al, 1983). The interface between the echogenic skin and the hypoechoic subcutis has been shown to be clearly depicted in most areas of the body, allowing measurement of the skin thickness, although with less accuracy than at higher frequencies. However, in oedematous skin or inflamed tissues using a 7.5- or 10-MHz transducer, it has been shown that the skin is markedly thickened and its echogenicity usually decreased with the interface between the dermis and subcutaneous fat often blurred (Fornage et al, 1995). I did not find any significant differences in skin layer thickness between normal controls and patient groups (figure 3.5.1) though the C_{4b} group had much higher values present but with a wide scatter of the data points. There was a significant increase in the subcutaneous layer thickness between normal controls and patient groups which have clinical evidence of tissue oedema present (figure 3.5.2) possibly reflecting increased interstitial tissue spaces.

There was no significant correlation between the skin and subcutaneous layer thickness and the X_0 and X_I-X_0 parameters obtained from tissue tonometry (figures 3.5.3 to 3.5.8; table 3.5.1). This may reflect the difficulty in making reliable measurements of tissue thickness in liposclerotic skin where the tissue boundaries are indistinct. The skin is more echogenic and sharply demarcated from the underlying hypoechoic subcutaneous fat making the measurement of the skin thickness easy in normal subjects with no significant evidence of oedema or inflammatory disease. Lipodermatosclerosis (LDS) generally comprises of tissue induration and a sharp inflammatory phase (Schmeller et al, 1992 book) and in the chronic phase, the inflammation of the tissues is manifested by increased echogenicity which is equal to that of the superficial underlying aponeural layer making it practically indistinguishable from the acute phase with the tissue echogenicity remaining dense. I therefore looked at the correlation between the tonometry parameters and the ultrasound-derived measurements of skin and subcutaneous layer thickness in the C_{4b} groups alone as compared to the non-liposclerotic groups, C_0, C_2 and C_{4a}. Again, there was no significant correlation present (table 3.5.1).

No association between skin or subcutaneous thickness measurements and any other marker of the severity of venous disease could be shown.

3.5.6. Conclusions

Ultrasound measurements of skin thickness and subcutaneous tissue thickness using a 7.5 MHz linear array probe showed no significant association with other markers of the severity of skin damage, especially in patients with the more severe forms of venous disease. There was no significant correlation between the ultrasound measurements and tissue tonometry parameters, which were previously found to correspond well with the clinical severity of the skin damage.

3.6. STUDY VI

The use of tissue tonometry in the assessment of the effects of compression stocking wear on the skin compliance in patients with lower limb chronic venous disease.

3.6.1. Rationale

The clinical sequelae of lower limb chronic venous disease includes oedema and lipodermatosclerosis. The site that is mainly affected is in the lower third of the lower limb, mainly in the medial aspect of the gaiter area (Callam et al, 1992). Lipodermatosclerosis has been described as a spectrum disease characterised by skin induration and hyperpigmentation (Kirsner et al, 1993). It has been previously shown that there is a correlation between the healing of venous ulcers and the degree of induration (Nemeth et al, 1989). The treatment of patients with lipodermatosclerosis have also included the use of compression stockings (Burnand et al, 1980).

Current methods of quantifying lower limb oedema include water displacement volumetry which has been shown to be a sensitive method and is often used as the 'gold standard' but this method is very messy and time consuming. The disc model method which is a circumferential measurement using a tape measure is another method that is used to quantify the limb volume. This method has been shown to have a very good correlation with water displacement volumetry ($r = 0.99$) (Kaulesar-Sukul et al, 1993).

The effects of compression therapy on the mechanical properties of the skin has never been objectively investigated. In this study, the effects of compression stocking wear on the mechanical properties of the skin in patients with lower limb chronic venous disease and the effect on the limb volume would be assessed by tissue tonometry and a circumferential measurement method respectively.

3.6.2. Aims

To objectively measure the effects of compression stockings in lower limb chronic venous disease using a tissue tonometer to assess the skin compliance.

The use of a circumferential method to assess the limb volume before and after compression stocking wear.

To assess the outcome of compression treatment on the displacement versus time curves obtained from the tissue tonometer.

To assess the effects of compression treatment on the spring and dashpot constants calculated from the application of the Kelvin-Hooke model.

3.6.3. Methodology

Sixteen limbs of eight patients (3M:5F; mean age of 66.6 years; range 58-75 years) referred from the out-patients clinic to the vascular laboratory for non-invasive venous studies were investigated. All the subjects had a full non-invasive venous assessment with duplex ultrasound to confirm the presence of venous disease and document the extent of the disease. Resting ABPI were also measured to confirm the absence of any arterial component with indices greater than or equal to 0.9. Subjects with evidence of peripheral arterial disease, diabetes, cardiac or renal disease were excluded from the study. The patients were investigated by tissue tonometry measurements and circumferential limb volume measurements. Informed consent was obtained from all the patients prior to the investigations. None of them had previously worn any form of compression stockings at least 6 months prior to the study. Three of the 8 patients were already on the waiting list for varicose vein surgery within the next 6 months.

They were all classified according to the CEAP method of classification by the author with the modification made to the C_4 group applied. Of the 16 limbs, 7 limbs were classified as C_3 (varicose veins with oedema) and 9 limbs were classified as C_{4b} (skin changes with palpable induration and no previous ulcers).

At the initial visit, week 0, all the patients were assessed in the supine position with the tissue tonometry measurements made in the gaiter area. The tonometer calibration was checked before and after each measurement. Three different sites were measured in all the limbs (at 6cm, 10cm and 12cm above the medial malleolus) and the mean value obtained for analysis. The limb volumes were measured by the disc model method.

A specially calibrated stand, designed by the author was used to mark the tonometry sites and the circumferential limb volume sites for reproducibility in sequential measurements.

After the tonometry measurements and circumferential limb volume measurements had been made, class 2 compression stockings (Venosan 2002, Saltzman, Switzerland) were prescribed and the patients measured in the surgical appliances department. They were instructed to wear it at all times except for when in bed. They were instructed to return in 6 weeks time when the tissue tonometry measurements and circumferential limb volume measurements would be repeated.

To ensure that the same tonometry sites and disc sites were measured on the second visit after 6 weeks, the specially calibrated stand was used to re-locate the previous tonometry and disc sites by using the previously noted heights on the column and the location of the big toe and heel in relation to the legs of the tripod stand. The tissue tonometry parameters and limb volume parameters measurements were repeated as in the previous visit.

Data Analysis

Displacement versus time curves were obtained from the tissue tonometry measurements and the distance and rate constant parameters were calculated by the system software for all three phases.

The spring and dashpot constants were also calculated from the Kelvin-Hooke model.

Medians and inter-quartile ranges were calculated and used as descriptors and the Wilcoxon ranked sums test was applied to test for statistical significance between the limb volume measurements by the disc model method and the distance parameter, rate constant parameter, spring and dashpot constants for all three phases obtained with the tissue tonometer after compression stocking wear.

3.6.4. Results

Tissue tonometry

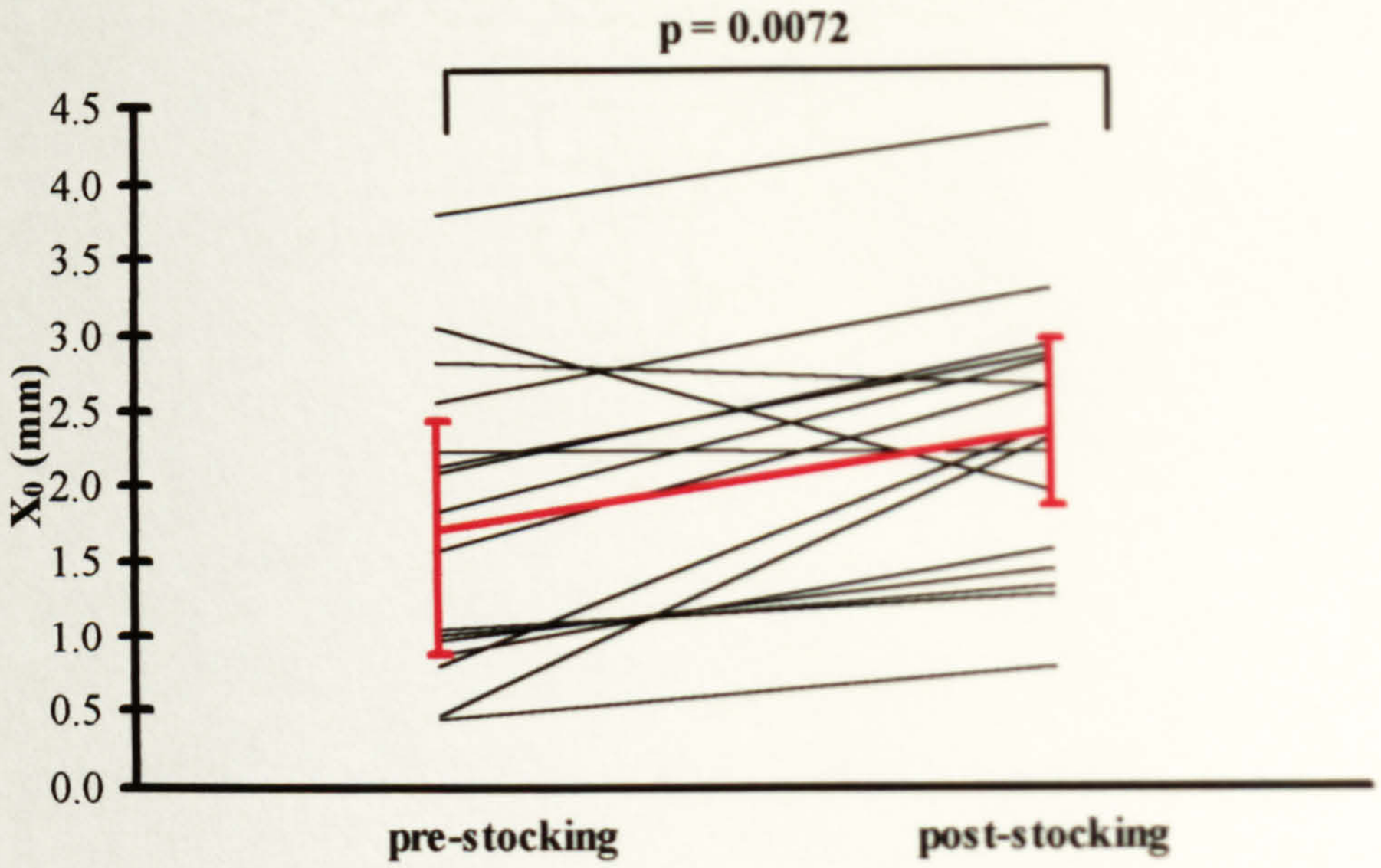


Figure 3.6.1 Distance travelled during the initial fast indentation phase I, which reflects the skin compliance, before and after compression stocking wear.

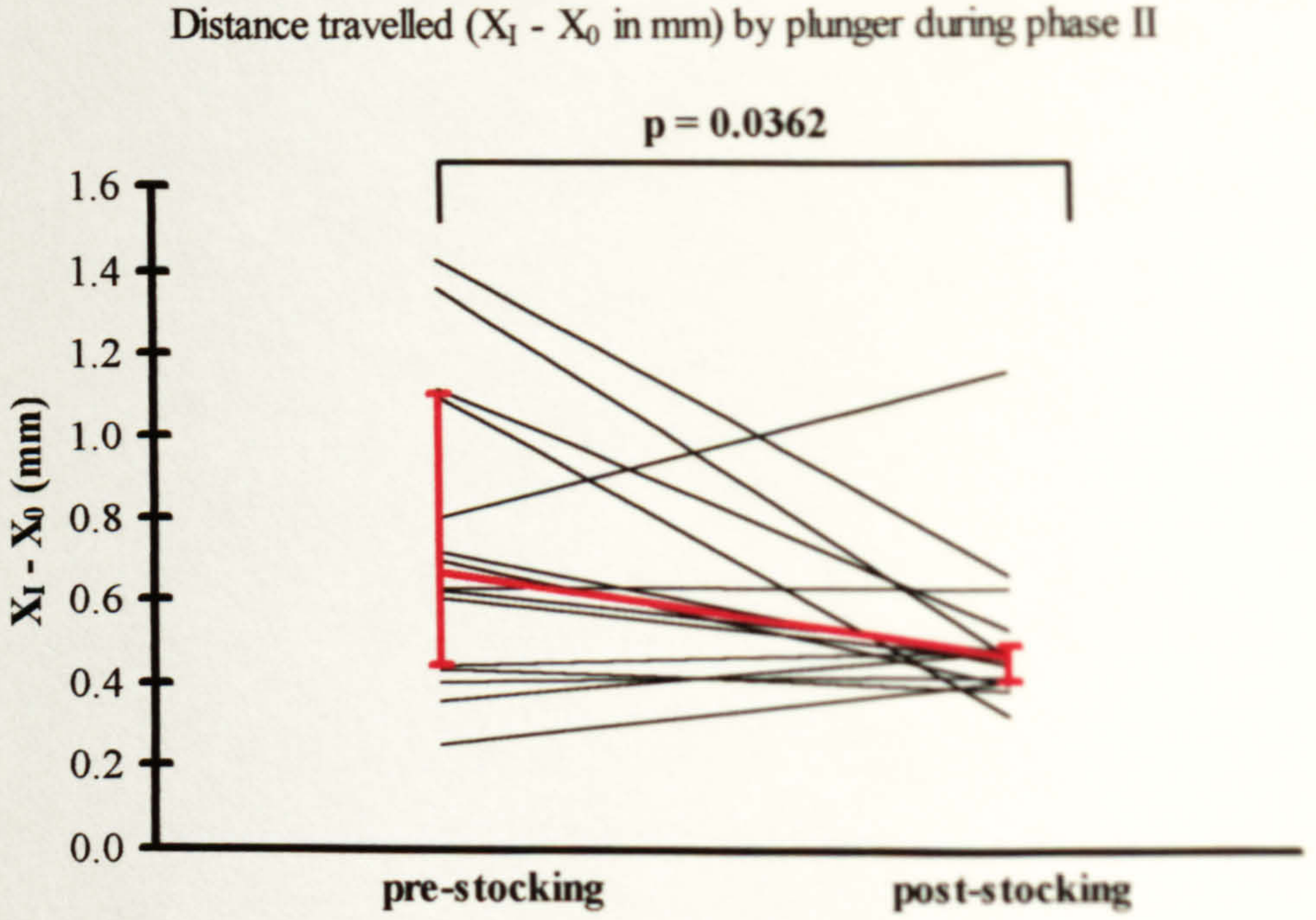


Figure 3.6.2 Distance travelled during the slower indentation phase II, before and after compression stocking wear.

Spring constant (C_2) of the Hooke element during phase I

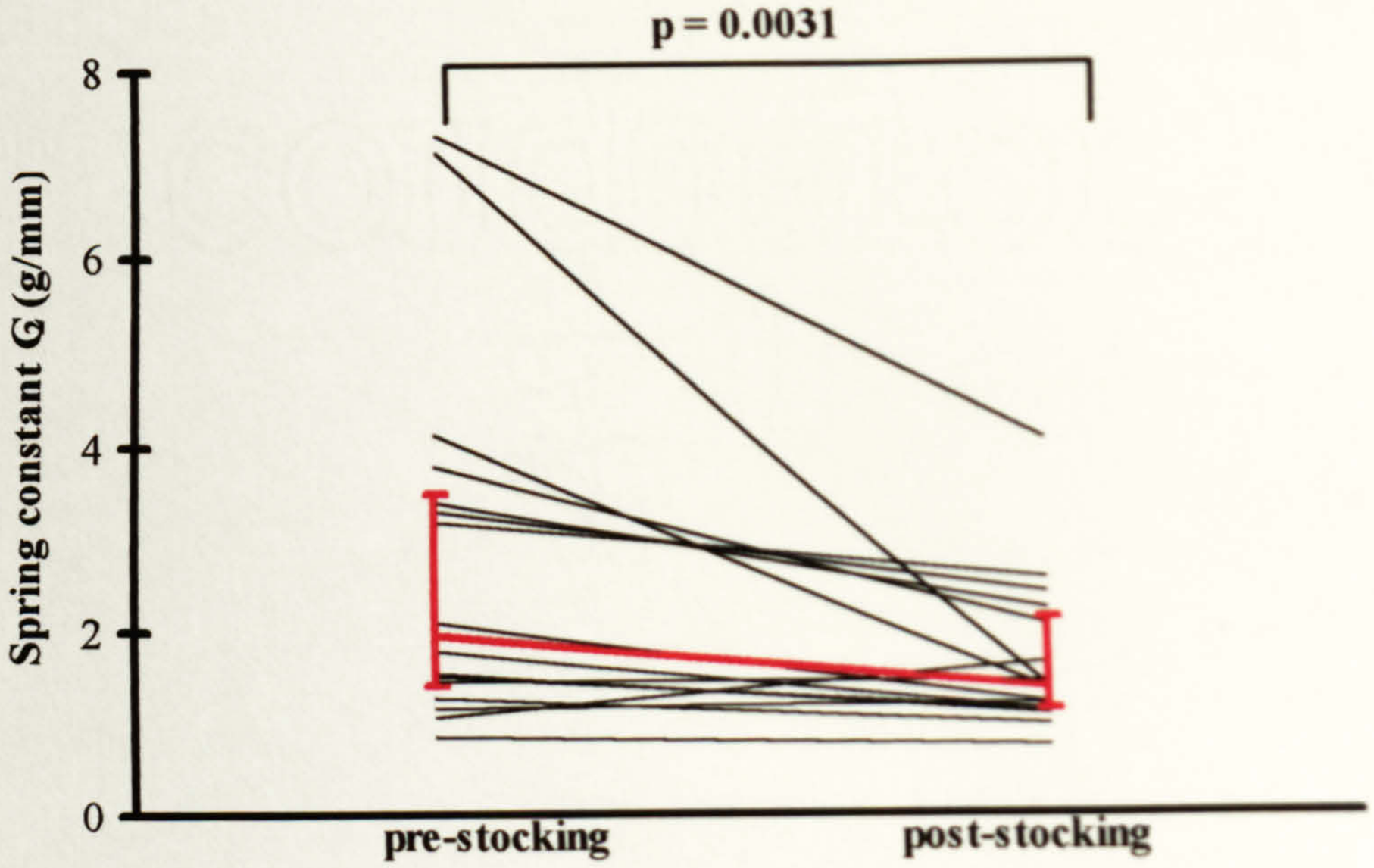


Figure 3.6.3 Spring constant C_2 during the initial fast indentation phase I, before and after compression stocking wear.

Circumferential limb volume by the disc model method

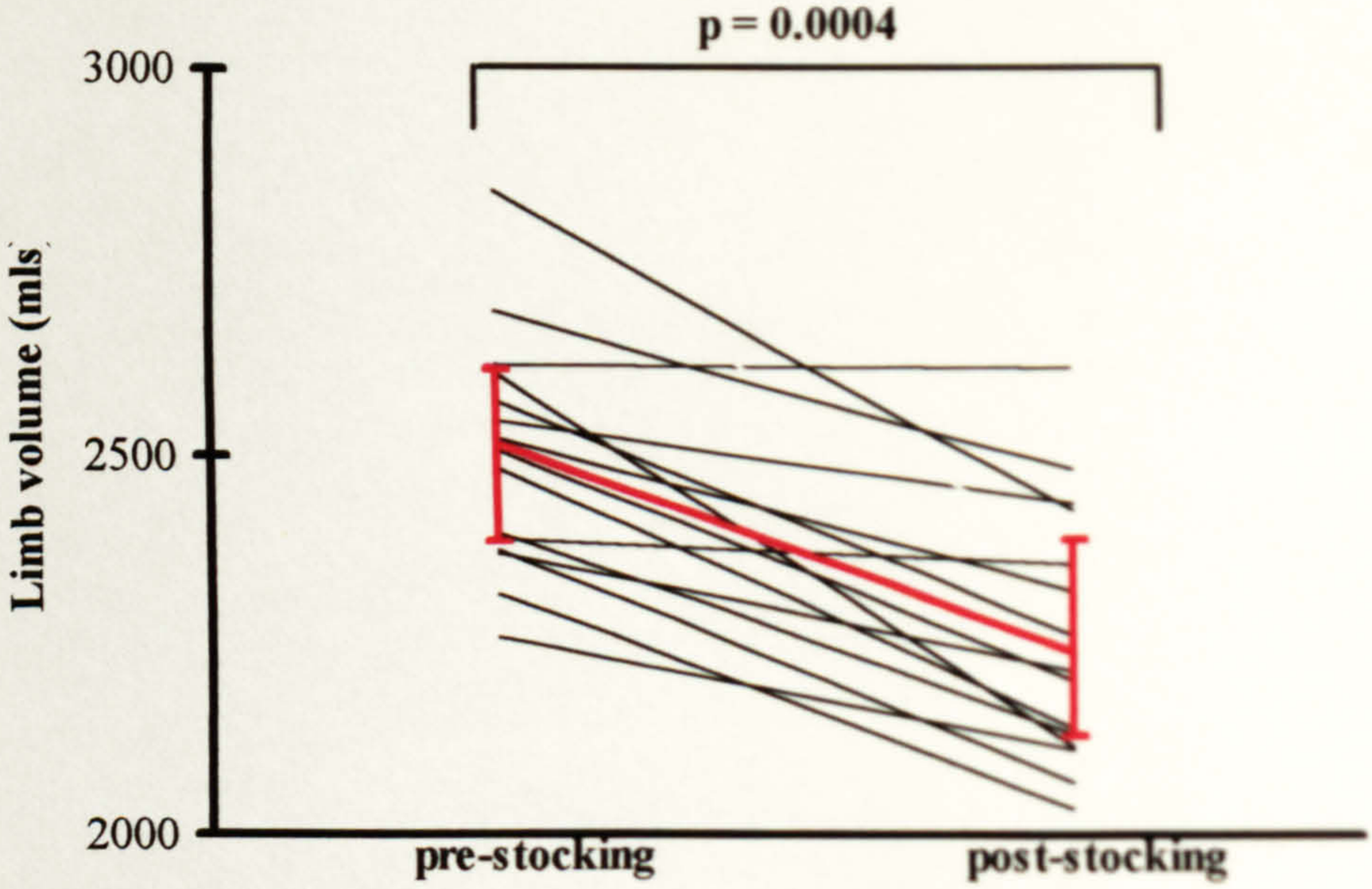


Figure 3.6.4 Circumferential limb volume as measured by the disc model method, before and after compression stocking wear.

	Phase I		Phase II				Phase III			
	X_O (mm)	X_O (mm)	X_I-X_O (mm)	X_I-X_O (mm)	-1/Tau (secs)	-1/Tau (secs)	X_I-X_O (mm)	X_I-X_O (mm)	-1/Tau (secs)	-1/Tau (secs)
	pre-	post-	pre-	post-	pre-	post-	pre-	post-	pre-	post-
median	1.68	2.34	0.66	0.46	2.44	3.07	0.60	0.50	73.92	65.55
LQ	0.94	1.52	0.44	0.40	2.27	2.52	0.20	0.35	61.20	45.45
UQ	2.31	2.84	1.10	0.48	3.03	4.20	0.83	0.73	115.3	76.36

Table 3.6.1 Median and interquartile values of the tissue tonometry parameters of the mechanical properties of the skin in patients with lower limb chronic venous disease before and after compression stocking wear.

	C_2	C_1	k	C_1	k
	(spring constant)	(spring constant)	Dashpot constant	(spring constant)	Dashpot constant
	Phase I	Phase II	Phase II	Phase III	Phase III
median	1.97	5.00	11.89	5.56	400.08
LQ	1.43	3.00	8.77	3.97	237.14
UQ	3.53	7.52	17.54	16.32	2043.97

Table 3.6.2 Median and inter-quartile range values for spring and dashpot constant parameters before compression stocking wear in the same group of patients in table 3.6.1.

	C_2	C_1	k	C_1	k
	(spring constant)	(spring constant)	Dashpot constant	(spring constant)	Dashpot constant
	Phase I	Phase II	Phase II	Phase III	Phase III
median	1.40	7.23	23.92	6.58	515.40
LQ	1.16	6.83	19.60	4.54	193.44
UQ	2.17	8.28	34.80	9.60	808.76

Table 3.6.3 Median and inter-quartile range values for spring and dashpot constant parameters after compression stocking wear in the same group of patients in table 3.6.1.

	Phase I		Phase II				Phase III			
	X_O (mm)	X_O (mm)	X_I-X_O (mm)	X_I-X_O (mm)	-1/Tau (secs)	-1/Tau (secs)	X_I-X_O (mm)	X_I-X_O (mm)	-1/Tau (secs)	-1/Tau (secs)
	pre-	post-	pre-	post-	pre-	post-	pre-	post-	pre-	post-
p value	0.007		0.04		0.19		0.74		0.12	

Table 3.6.4 p values obtained from the Wilcoxon ranked sums test to test for statistical significance between tissue tonometry parameters before and after compression stocking wear.

	Phase I		Phase II				Phase III			
	C_2	C_2	C_1	C_1	k	k	C_1	C_1	k	k
	pre-	post-	pre-	post-	pre-	post-	pre-	post-	pre-	post-
p value	0.003		0.06		0.03		0.57		0.41	

Table 3.6.5 p values obtained from the Wilcoxon ranked sums test to test for statistical significance between the spring and dashpot constants obtained from the Kelvin-Hooke model before and after compression stocking wear.

There was a significant increase in the distance travelled, X_0, during phase I ($p = 0.007$) after 6 weeks of compression stocking wear (figure 3.6.1). The distance travelled, X_I-X_0, during phase II after stocking wear decreased significantly ($p = 0.036$) (figure 3.6.2). Interestingly, in the distance travelled, X_I-X_0, during phase III, there was no significant difference in this parameter after stocking wear ($p = 0.74$) (table 3.6.4). There was also no significant difference in the rate constant parameters during phases II and III after stocking wear (table 3.6.4).

There was a significant difference in the spring constant parameter of the Hooke element, C_2, after compression stocking wear ($p = 0.003$) (table 3.6.5).

In phase II, the C_1 and k parameters were significantly increased after stocking wear ($p = 0.06$; $p=0.03$) (table 3.6.5) However, during phase III, there were no significant differences in the C_1 and k parameters after stocking wear (table 3.6.5).

After 6 weeks of compression stocking wear the median fall in limb volume was significantly reduced (median fall in limb volume = 273.3 mls; $p = 0.0004$) (figure 3.6.4).

3.6.5. Discussion

Compression treatment has been used since at least the time of Hippocrates and remains the most effective non-operative treatment for patients with lower limb chronic venous disease and venous ulceration. A study reporting the results of using compression therapy in the management of venous ulcers showed that there was a 97% healing of ulcers in patients who were compliant with stocking use as compared to 55% who were non-compliant ($p< 0.0001$) (Mayberry et al, 1991). Patient compliance using compression therapy has been a major criticism as some patients may be initially intolerant of compression in areas of hypersensitivity adjacent to an active ulcer or at sites of previously healed ulcers. Another potential hazard of compression therapy is the exacerbation of underlying arterial insufficiency. It has been recommended that patients with ulcers of mixed aetiology including arterial insufficiency should not be given compression therapy (Callam et al, 1987).

The exact mechanism by which compression therapy works remains unknown. Investigators using invasive and non-invasive techniques have not been able to show significant changes in ambulatory venous pressure or venous recovery times with the use of compression therapy (Mayberry et al, 1991). Others have shown significant trends

towards improvement on the venous haemodynamics using air plethysmography with compression stockings (Ibegbune et al, 1997).

Other reported mechanisms suggest that compression therapy has a direct effect on subcutaneous pressure with the supine peri-malleolar subcutaneous pressures increasing with elastic compression in limbs with chronic venous disease (Nehler et al, 1993). Improvements in skin and subcutaneous tissue microcirculatory haemodynamics may contribute to the benefits of compression therapy. It has been demonstrated by laser Doppler fluxmetry that there is cutaneous hyperaemia in patients with venous disease but not in normal controls (Christopoulos et al, 1991). This was reduced by compression treatment for 30 minutes.

Previous studies using mechanical impedance measurements has shown that in oedematous tissues the skin becomes stretched and less distensible and with the reduction of the oedema, distensibility tended towards normal values (Sodeman and Burch, 1983). The use of compression stockings have been shown to cause a decrease in leg volume (Kakkar et al, 1982; Pierson et al, 1983) in patients with lower limb oedema. In this study patients were treated for a relatively short period using class II compression hosiery. This treatment is widely prescribed in the management of chronic venous disease, although it is only in recent years that objective assessment of the efficacy of this line of management has been undertaken. Clinicians who use such treatments are aware that rapid improvements in ulcers and damaged skin may be observed. But could these changes be measured objectively?

There was a significant increase in the distance travelled by the plunger of the tonometer amounting to an increase of about one third in X_0, during phase I ($p = 0.007$) demonstrating that the skin compliance had improved (figure 3.6.1). The distance travelled, X_I-X_0, during phase II after stocking wear decreased significantly ($p = 0.036$) indicating that the amount of tissue fluid present beneath the plunger had decreased (figure 3.6.2). Interestingly, in the distance travelled, X_I-X_0, during phase III, there was no significant difference in this parameter after stocking wear ($p = 0.74$) (table 3.6.4). It is possible that although the oedema was decreased in the skin of this group, resulting in reduced distance travelled by the plunger, the improvement in tissue compliance caused an increase. The two effects may have cancelled each other out. There was also no significant difference in the rate constant parameters during phases II and III after stocking wear (table 3.6.4).

There was a significant difference in the spring constant parameter of the Hooke element, C_2, after compression stocking wear ($p = 0.003$) (figure 3.6.3). The C_2 parameter decreased significantly and as this constant is a measure of the initial ability of the skin to undergo elastic deformation after force had been applied to it and the lower the value, the better the compliance of the skin.

The spring constant, C_1, of the Kelvin element and the dashpot constant, k, provide a measure of the subsequent deformation, X_I-X_0, for a given load. This gives a measure of the amount of tissue oedema present. As they are inversely related , the C_1 and k parameters would be lower if the distance travelled by the plunger in the viscous creep phase increases and vice versa. In phase II, the C_1 and k parameters were significantly increased after stocking wear ($p = 0.06$; $p = 0.03$) (tablee 3.6.5) as a result of the decrease in the X_I-X_0 parameter (table 3.6.1) indicating reduced tissue fluids beneath the plunger. However, during phase III, there were no significant differences in the C_1 and k parameters after stocking wear (table 3.6.5).

The results shown by tissue tonometry could have been attributable to measurements being made in different positions on the limb from on the first occasion. However, great care was taken to avoid this by the use of a device to ensure that all measurements were made at the same level above the sole of the foot on each occasion. Had the location of measurement been different, the observations would have included much more variance. In fact, for the phase I distance, all except three observations showed a change in the same direction, confirming that a real effect of compression treatment had been observed.

After 6 weeks of compression stocking wear, the median fall in limb volume was 273 ml as measured by the disc model method, about a 10% fall in limb volume. This finding is consistent with the expected influence of compression stockings on the Starling equilibrium between intravascular and extracellular compartments, reducing limb oedema. Measurements were made immediately after removing the stocking at the second visit so that the changes in volume may have also been attributable to a reduction in the venous volume (the volume of blood in the small and large veins). This is suggested by the reduced distance parameter during phase II, which probably mainly reflects the volume of blood in the veins.

The measurements reported here show that very distinct changes in the mechanics of the skin and limb volume can be measured in patients following a short period of compression treatment.

3.6.6. Conclusion

The response to compression treatment used for 6 weeks can be readily assessed by tissue tonometry. This technique is sensitive enough to show changes in skin compliance as well as indicating some reduction in the volume of the venous compartment. Circumferential limb volumetry was used to confirm that compression treatment had also influenced a conventional measure of the efficacy of treatment and showed a 10% reduction in limb volume following treatment.

3.7. STUDY VII

The CEAP method of classification and scoring of lower limb venous disease - ease of use and inter-examiner variability.

3.7.1. Rationale

In the past, research into lower limb chronic venous disease had relied mainly on the clinical appearance of the clinical sequelae of the disease such as varicose veins, skin changes and ulceration without any objective methods of testing of the venous system to confirm the diagnosis. This has led to non-uniform diagnosis between centres resulting in poor correlation of results between the different treatment practices in lower limb chronic venous disease.

The CEAP system of classification was introduced because there was a need for uniform diagnosis and meaningful scientific communication between research centres.

The methods of classification, grading and reporting of lower limb venous disease used throughout in this thesis are based on the recently devised **C**linical, (A)Etiological, **A**natomical and **P**athophysiological method (CEAP) of classification and grading of lower limb chronic venous disease.

The CEAP classification presents a new level of diagnosis in lower limb chronic venous disease and has been criticised for being too complex as it requires more detailed analysis of the disease involving several tables (Beebe et al, 1995). However, the requirements for the CEAP method of classification are similar to those used for diagnosing peripheral arterial disease, which has been in use for some time. A feature of the CEAP method of classification of lower limb chronic venous disease is the scoring system of chronic venous dysfunction that is incorporated within the system, based on the thought that though the grading of symptoms is subjective, the grading of signs is objective. This provides a numerical base for the scientific comparison of limb condition and evaluation of results of treatment based on the number of anatomic segments affected (anatomic score), grading of symptoms and signs (clinical score) and disability (disability score).

The initial evaluation of patients with suspected chronic venous disease relies on the history taking and physical examination of the patient. A patient presenting with symptoms that may be related to venous disease should have a non-invasive test to confirm or exclude the presence of venous dysfunction.

In order to assess the ease of use of this classification system, I have measured the inter-variability between different examiners with varying degrees of experience in the field, by applying the *kappa* measure of agreement between the examiners as described by Cohen et al in 1960.

3.7.2. Aim

To assess the inter-variability between examiners and ease of use of the application of the CEAP method of classification and scoring of lower limb venous disease.

3.7.3. Methodology

Twenty eight limbs of 14 patients (6M:8F; mean age of 56.6 years; age range: 25 -75 yrs) referred to the vascular laboratory for non-invasive venous assessment of their limbs were classified and scored for lower limb venous disease according to the CEAP method.

Each limb was classified and scored for the disease by three different examiners with varying degrees of expertise in the field with their results remaining unknown to each other. Examiner A is an experienced vascular surgeon (senior specialist registrar in vascular surgery) who is also a research fellow in the vascular laboratory. Examiner B, the author, is an accredited vascular technologist with 6 years experience in the duplex ultrasound examination of lower limb venous disease. Examiner C is a trainee vascular technologist with 1 year experience of lower limb venous investigations, which also includes duplex ultrasound examination.

The method of clinical classification and whether the patient is symptomatic or asymptotic, required all three examiners to take a history and examine the patient clinically, following the strict guidelines of this method of classification. However, due to the nature of the CEAP method of classification, the other classification details of aetiology, anatomy and pathophysiology could only be completed using information obtained from duplex ultrasound scanning. In these cases, only one examiner (examiner B) performed the duplex ultrasound investigation and produced a standard report from which the other examiners could complete their classification of the disease. This was necessary as examiner B was the most experienced in duplex ultrasound scanning thus avoiding the variations that may occur between different examiner's reports.

Similarly, for the scoring of the disease, the clinical score and disability score were obtained from direct correspondence with the patient, following the strict guidelines of

this method of scoring. For the anatomical score, again, the examiners had to acquire their information from the report produced by examiner B after duplex ultrasound scanning. The data from all three examiners were examined and the *kappa* measure of agreement was applied to compare the inter-examiner variability between the three examiners using examiner A, the clinician and vascular surgeon, as the reference standard.

Statistical analysis

The method for the *kappa* measure of agreement is used for analysis and is described in details in see section 2.11.

The maximum value of *kappa* is 1, which represents perfect agreement, and *kappa* will take the value zero if there is only chance agreement.

3.7.4. Results

			CEAP Method of classification				**CEAP Method of scoring**		
Pt No	Clinical	A/S	Etiologic	Anatomic	Pathophysiologic	Clinical	Disability	Anatomic	Examiner
1	C_0	A	E	A	P	0	0	0	A
1	C_0	A	E	A	P	0	0	0	B
1	C_0	A	E	A	P	0	0	0	C
1	C_0	A	E	A	P	0	0	0	A
1	C_0	A	E	A	P	0	0	0	B
1	C_0	A	E	A	P	0	0	0	C
2	C_0	A	E	A	P	0	0	0	A
2	C_0	A	E	A	P	0	0	0	B
2	C_0	A	E	A	P	0	0	0	C
2	C_0	A	E	A	P	0	0	0	A
2	C_0	A	E	A	P	0	0	0	B
2	C_0	A	E	A	P	0	0	0	C
3	C_0	A	E	A	P	0	0	0	A
3	C_0	A	E	A	P	0	0	0	B
3	C_0	A	E	A	P	0	0	0	C
3	C_0	A	E	A	P	0	0	0	A
3	C_0	A	E	A	P	0	0	0	B
3	C_0	A	E	A	P	0	0	0	C
4	C_0	A	E	A	P	0	0	1	A
4	C_1	A	E	A	P	0	0	1	B
4	C_0	A	E	A	P	0	0	1	C
4	C_1	A	E	A	P	0	0	1	A
4	C_1	A	E	A	P	0	0	1	B
4	C_1	A	E	A	P	0	0	1	C
5	C_2	S	E_P	$A_{S,P}$	P_R	1	1	3	A
5	C_2	S	E_P	$A_{S,P}$	P_R	1	1	3	B
5	C_2	S	E_P	$A_{S,P}$	P_R	1	1	3	C
5	C_2	S	E_P	$A_{S,P}$	P_R	1	1	2	A
5	C_2	S	E_P	$A_{S,P}$	P_R	1	1	2	B
5	C_2	S	E_P	$A_{S,P}$	P_R	1	1	2	C
6	C_2	S	E_P	A_S	P_R	1	1	3	A
6	C_2	S	E_P	A_S	P_R	1	1	3	B

			CEAP Method of classification				CEAP Method of scoring		
Pt No	Clinical	A/S	Etiologic	Anatomic	Pathophysiologic	Clinical	Disability	Anatomic	Examiner
6	C_2	S	E_P	A_S	P_R	1	1	3	C
6	C_3	S	E_P	A_S	P_R	3	1	3	A
6	C_3	S	E_P	A_S	P_R	3	1	3	B
6	C_3	S	E_P	A_S	P_R	3	1	3	C
7	C_3	A	E_P	A_S	P_R	1	0	3	A
7	C_3	A	E_P	A_S	P_R	1	0	3	B
7	C_2	A	E_P	A_S	P_R	0	0	3	C
7	C_3	S	E_P	A_S	P_R	2	1	4	A
7	C_3	S	E_P	A_S	P_R	2	1	4	B
7	C_2	S	E_P	A_S	P_R	1	1	4	C
8	C_3	S	E_P	A_S	P_R	2	1	2	A
8	C_3	S	E_P	A_S	P_R	2	1	2	B
8	C_2	S	E_P	A_S	P_R	1	1	2	C
8	C_3	S	E_P	A_S	P_R	2	1	2	A
8	C_3	S	E_P	A_S	P_R	2	1	2	B
8	C_2	S	E_P	A_S	P_R	1	1	2	C
9	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	6	A
9	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	6	B
9	C_3	S	E_S	$A_{S,P,D}$	P_R	3	2	6	C
9	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	7	A
9	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	7	B
9	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	7	C
10	C_4	S	E_S	$A_{S,P,D}$	P_R	4	2	4	A
10	C_4	S	E_S	$A_{S,P,D}$	P_R	4	2	4	B
10	C_4	S	E_S	$A_{S,P,D}$	P_R	4	2	4	C
10	C_4	S	E_P	A_S	P_R	4	2	3	A
10	C_4	S	E_P	A_S	P_R	4	2	3	B
10	C_4	S	E_P	A_S	P_R	4	2	3	C
11	C_4	S	E_P	A_S	P_R	7	2	2	A
11	C_4	S	E_P	A_S	P_R	6	2	2	B
11	C_4	S	E_P	A_S	P_R	6	2	2	C
11	C_4	S	E_P	A_S	P_R	7	2	2	A
11	C_4	S	E_P	A_S	P_R	7	2	2	B
11	C_4	S	E_P	A_S	P_R	7	2	2	C
12	C_6	S	E_S	$A_{S,D}$	P_R	12	2	5	A
12	C_6	S	E_S	$A_{S,D}$	P_R	12	2	5	B
12	C_6	S	E_S	$A_{S,D}$	P_R	10	2	5	C
12	C_6	S	E_P	A_S	P_R	11	2	2	A
12	C_6	S	E_P	A_S	P_R	11	2	2	B
12	C_6	S	E_P	A_S	P_R	11	2	2	C
13	C_4	S	E_P	A_S	P_R	7	2	2	A
13	C_4	S	E_P	A_S	P_R	7	2	2	B
13	C_4	S	E_P	A_S	P_R	7	2	2	C
13	C_4	S	E_P	A_S	P_R	7	2	2	A
13	C_4	S	E_P	A_S	P_R	7	2	2	B
13	C_4	S	E_P	A_S	P_R	7	2	2	C
14	C_5	S	E_P	A_S	P_R	8	2	2	A
14	C_5	S	E_P	A_S	P_R	8	2	2	B
14	C_5	S	E_P	A_S	P_R	8	2	2	C
14	C_5	S	E_P	A_S	P_R	10	2	2	A
14	C_5	S	E_P	A_S	P_R	10	2	2	B
14	C_4	S	E_P	A_S	P_R	8	2	2	C

Table 3.7.1 Inter-variability of the ceap method of classification and scoring of lower limb venous disease between different examiners.

	Examiner A							
	CEAP method of classification					*CEAP method of scoring*		
	Clinical	Etiological	A/S	Anatomical	Patho-physiological	Clinical	Disability	Anatomical
Examiner B	0.875	1.00	1.00	1.00	1.00	0.90	1.00	1.00
Examiner C	0.60	1.00	1.00	1.00	1.00	0.52	1.00	1.00

Table 3.7.2 Summary of the kappa measure of agreement between the different examiners.

There was perfect agreement between all three observers in all the parameters measured with the exceptions of the clinical methods of classification and scoring parameters (tables 3.7.1 and 3.7.2).

There was excellent agreement between examiners A and B in the clinical methods of classification and scoring parameters.

There was fair to good agreement between examiners A and C in the clinical methods of classification and scoring parameters.

3.7.5. Discussion

The aim of this study was to assess the ease of use of the CEAP method of classification. This involves use of both clinical skills and the interpretation of objective vascular laboratory tests in order to establish the complete classification of a patient. There was perfect agreement between examiners A and B in all the parameters assessed with the exception of the clinical classification and clinical scoring parameters which had excellent agreement. The disagreements lay in the clinical examination of patients and therefore it is not surprising that the person with the least experience of clinical skills fared least well in this part of the assessment. The perfect agreements between the classification parameters of aetiology, anatomy and pathophysiology and in the anatomical scoring parameter were expected as all three examiners relied on the accuracy of the duplex ultrasound report analysis produced by examiner B. The duplex ultrasound report provided details of the pathophysiology and anatomy of the limb under investigation and all the examiners had to do was follow the detailed instructions described in the CEAP method to classify and score the disease. This system if used by vascular surgeons would rely on vascular technologists to provide the aetiological, anatomical and pathophysiological aspects of the classification in addition to their clinical classifications to get the full picture.

The slight difference in the clinical classification parameter between examiners A and B arose as a result of examiner A not identifying the presence of some dermal flares in 1 limb (rt leg of pt no 4) which were identified by examiner B who had the advantage of close-up view of the limb while performing the duplex scan. The reason for the slight discrepancy in the clinical scoring parameter between examiners A and B was due to one of the limbs (rt leg of pt no 11) classified by examiner A as having moderate oedema whereas examiner B classified it as having significant oedema.

Between examiners A and C, there was also perfect agreement in all the parameters assessed with the exception of the clinical classification and clinical scoring parameters which had a fair to good agreement. For the same reasons as described above, the perfect agreement between the classification parameters of aetiology, anatomy and pathophysiology and in the anatomical scoring parameter was expected. However, the discrepancy in the clinical classification parameter between examiners A and C is most likely to be due to the lack of clinical experience of examiner C in identifying details of the disease. In 4 limbs, examiner C failed to clinically identify the presence of oedema, failed to identify presence of localised pigmentation in 2 limbs and classified 1 limb with previous episodes of ulceration as liposclerotic with no evidence of previous ulceration. These differences between examiners A and C appear largely due to the fact that examiner C, a trainee vascular technologist, did not appreciate the difference between moderate and significant in the clinical aspects of chronic venous disease. There were also some discrepancies in the clinical scoring between examiners A and C with examiner C underscoring in 7 limbs again reflecting a lack of clinical experience in assessing lower limb venous disease.

This study confirms that, in a limited range of patients, the parts of the CEAP classification based on vascular laboratory investigation are very reliable and depend exclusively on the accuracy of the vascular laboratory report. The repeatability of venous duplex ultrasound investigations was not tested in this investigation, although the results of this type of study have been reported elsewhere in the literature. The clinical classification and scoring depend greatly on the clinical skills of the examiner and experience is required to achieve consistent results in this type of examination.

3.7.6. Conclusions

The worry expressed by some authors (Darke and Ruckley, 1996) of this method of classification *'requiring feats of memory or constant reference to keys'* should be easy to overcome. It was envisaged as a method of categorising data when entered into computer databases, when used to its fullest extent. Data categorised in this way could then be used to compare the patients in clinical studies performed in centres separated by great distances. This study confirms in a simple way, that provided reliable clinical examination is performed by a skilled clinician and that vascular laboratory testing is performed by a trained vascular technologist, reliable classification of patients with venous disease can be obtained using the CEAP system.

3.8. STUDY VIII

Correlation of the CEAP method of classification and scoring of lower limb chronic venous disease with objective non-invasive methods of quantifying the clinical sequelae of the disease.

3.8.1. Rationale

The tissue tonometer developed in our laboratory has been shown to be a reliable and reproducible method of objectively assessing the skin changes in patients with lower limb chronic venous disease.

The quantification of venous reflux plays an important role in venous disease research. Techniques such as air plethysmography and duplex scanning are well established in quantifying reflux with the former providing a measure of total limb volume and the latter providing volume measurements in individual veins.

The majority of clinical investigations performed using air plethysmography involve patients with lower limb venous disease. The haemodynamic characteristics associated with progressive chronic venous disease have also been investigated with the parameters obtained from APG (Welkie et al, 1992).

It has been shown that the combination of air plethysmography and duplex ultrasound scanning provides the best means of assessing venous reflux (Bays et al, 1994) and for the complete evaluation of limbs with chronic venous disease (van Bemmelen et al, 1993).

3.8.2. Aims

To correlate duplex-derived parameters of lower limb venous reflux quantification with the clinical severity of the disease as classified by the CEAP method .

To correlate air plethysmography-derived parameters of venous function and arterial perfusion with the clinical severity of the disease as classified by the CEAP method.

To correlate photoplethysmography-derived parameters of venous function and arterial perfusion with the clinical severity of the disease as classified by the CEAP method.

To correlate the tissue tonometry-derived parameters of the mechanical properties of lower limb skin changes with the clinical severity of the disease as classified by the CEAP method.

To correlate the Doppler-derived resting ankle brachial pressure indices (ABPI) and APG-derived arterial perfusion within the CEAP groups.

Materials

The Acuson 128XP/10v (Acuson, Mountain View, California, USA) computed sonography system with ART (acoustic response technology) using a 5- or 7.5- MHz linear array transducer was used for all the measurements below.

A tissue tonometer designed and built by PDCS and standardised by JF.

A model PPG13 Vasculab® Photoplethysmograph (PPG) (Medasonics, Mountain View, California).

An air plethysmograph , APG-1000C (ACI Medical inc., Sun Valley, California).

A standardised rapid cuff inflation and deflation unit constructed in our laboratory (Middlesex Hospital Vascular Laboratory, 1995) with a calf cuff width of 12cm was used to augment flow in the vessels.

A sonic digitiser pen was used to calculate area measurements from the spectral Doppler display.

Methodology

Eighty two limbs of 41 subjects (18M:23F; mean age of 57.2 years; age range 21-90 years) referred to the vascular laboratory for non-invasive venous investigations were entered in the study. They all had an initial full standard non-invasive venous assessment at the Middlesex Hospital Vascular laboratory with colour duplex ultrasound scanning. All limbs were classified and scored according to the CEAP method of classification of lower limb venous disease with a section of the classification system modified by the author included in the classification. The CEAP modification was applied here to subdivide the classification group C_4 to separate patients with skin changes and no palpable induration into group $C_{4a,}$ from those with skin changes with palpable evidence of induration into group C_{4b}.

The inclusion criteria for normal subjects include limbs with no evidence of venous or arterial disease and for the patient group, limbs with evidence of venous disease. Patients with diabetes, cardiac disease, kidney disease or peripheral arterial disease of the lower limbs as demonstrated by a resting ABPI of < 0.90 were excluded from the study.

Subjects underwent full duplex ultrasound investigation of all the veins in the lower limb to document the extent of the disease for a full classification and scoring of the disease according to the CEAP method. Resting ankle brachial pressure indices were also measured to confirm presence or absence of any significant peripheral arterial disease The patient was invited to enter the study after patient consent had been obtained. On a second visit, the subject underwent 2 hours of non-invasive measurements which included air plethysmography, resting ankle brachial pressure indices, photoplethysmography, tissue tonometry and the Duplex ultrasound derived parameters.

Duplex ultrasound scanning to quantify the reflux volume flow with the following parameters measured from the B-mode image and the frozen spectral Doppler display: vessel diameter, time-averaged velocity and duration of reflux. Air plethysmography, resting ankle brachial pressure indices, photoplethysmography and tissue tonometry were carried out on all the above 82 limbs of selected patients and the correlation of the derived parameters with the clinical severity of the disease were analysed.

3.8.3. Results

The limbs of the subjects were clinically classified as follows:

Eight limbs of CEAP class C_0 (4M:4F; mean age of 33.2 yrs; age range 21-55 yrs);

Ten limbs of CEAP class C_1 (1M:9F; mean age of 52.4 yrs; age range 22-90 yrs);

Ten limbs of CEAP class C_2 (1M:9F; mean age of 51.9 yrs; age range 22-72 yrs);

Eleven limbs of CEAP class C_3 (2M:9F; mean age of 59.1 yrs; age range 44-90 yrs);

Thirteen limbs of CEAP class C_{4a} (9M:4F; mean age of 62.4 yrs; age range 40-83 yrs);

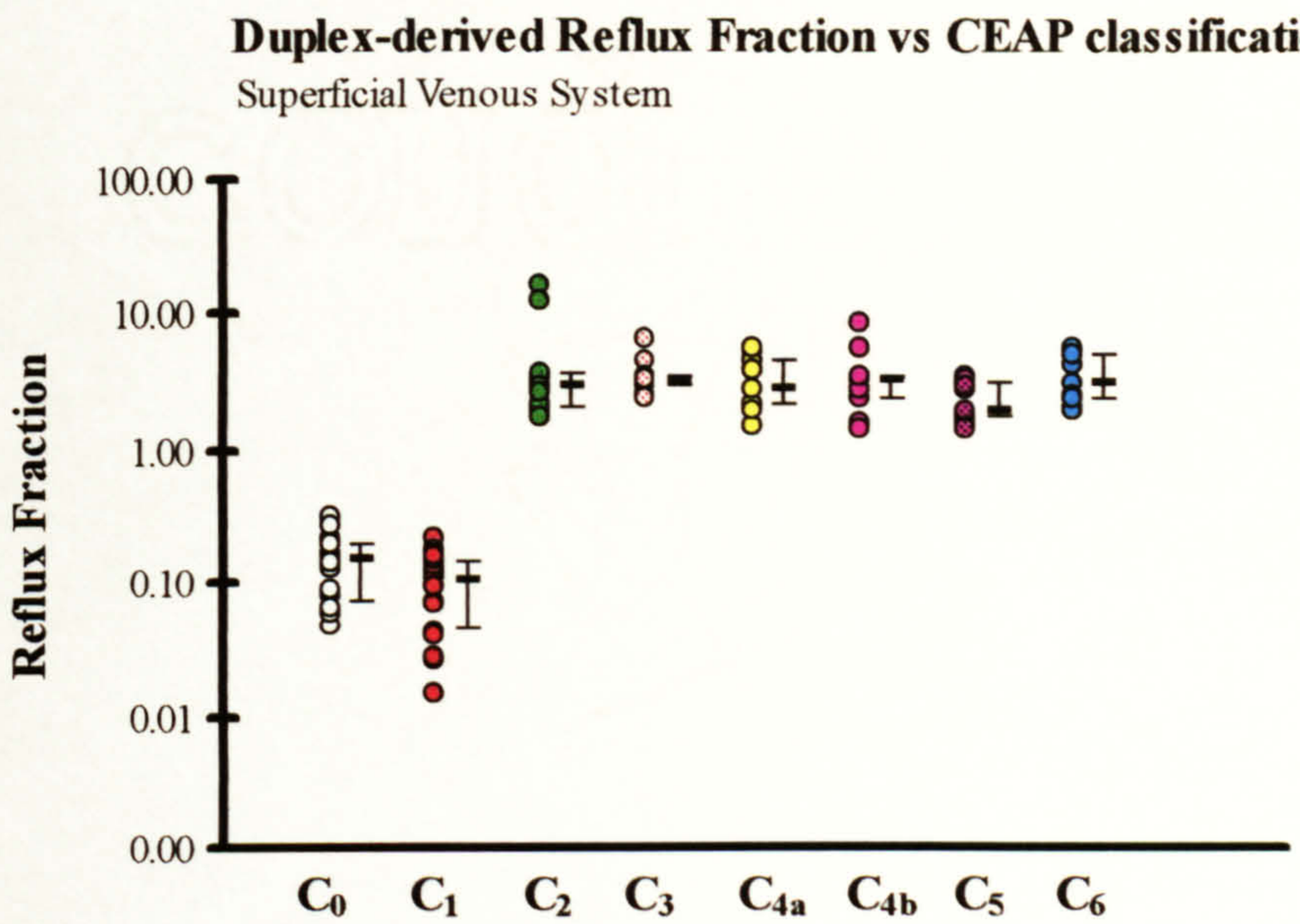
Ten limbs of CEAP class C_{4b} (1M:9F; mean age of 64.5 yrs; age range 50-75 yrs);

Ten limbs of CEAP class C_5 (5M:5F; mean age of 61.8 yrs; age range 50-71 yrs);

Ten limbs of CEAP class C_6 (8M:2F; mean age of 68.4 yrs; age range 22-90 yrs);

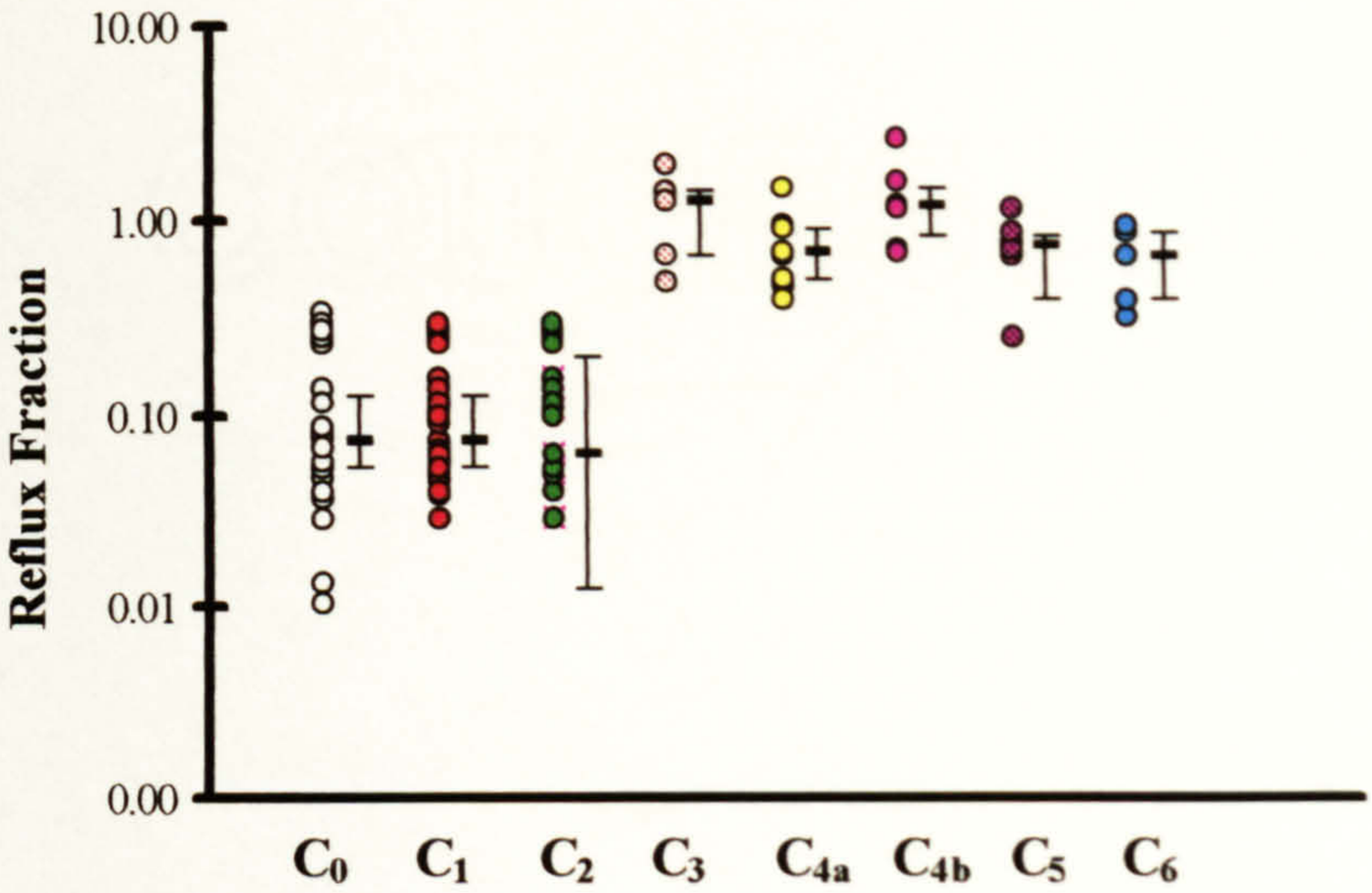
Of the 82 limbs, 54 (66%) were of the aetiology E_p and 13 (16%) of the aetiology E_s. Twenty five limbs (31%) were asymptomatic and 57 limbs (70%) were symptomatic. Reflux was distributed between the superficial, deep and perforator systems of the limbs as follows: 36 A_S (44%), A_D (0%), 2 A_P (2.4%), 7 $A_{S,D}$ (8.5%), 9 $A_{S,P}$ (11%), 2 $A_{P,D}$ (2.4%) and 11 $A_{S,P,D}$ (13%) and 15 with no evidence of reflux (18%).

Duplex-derived reflux parameters results



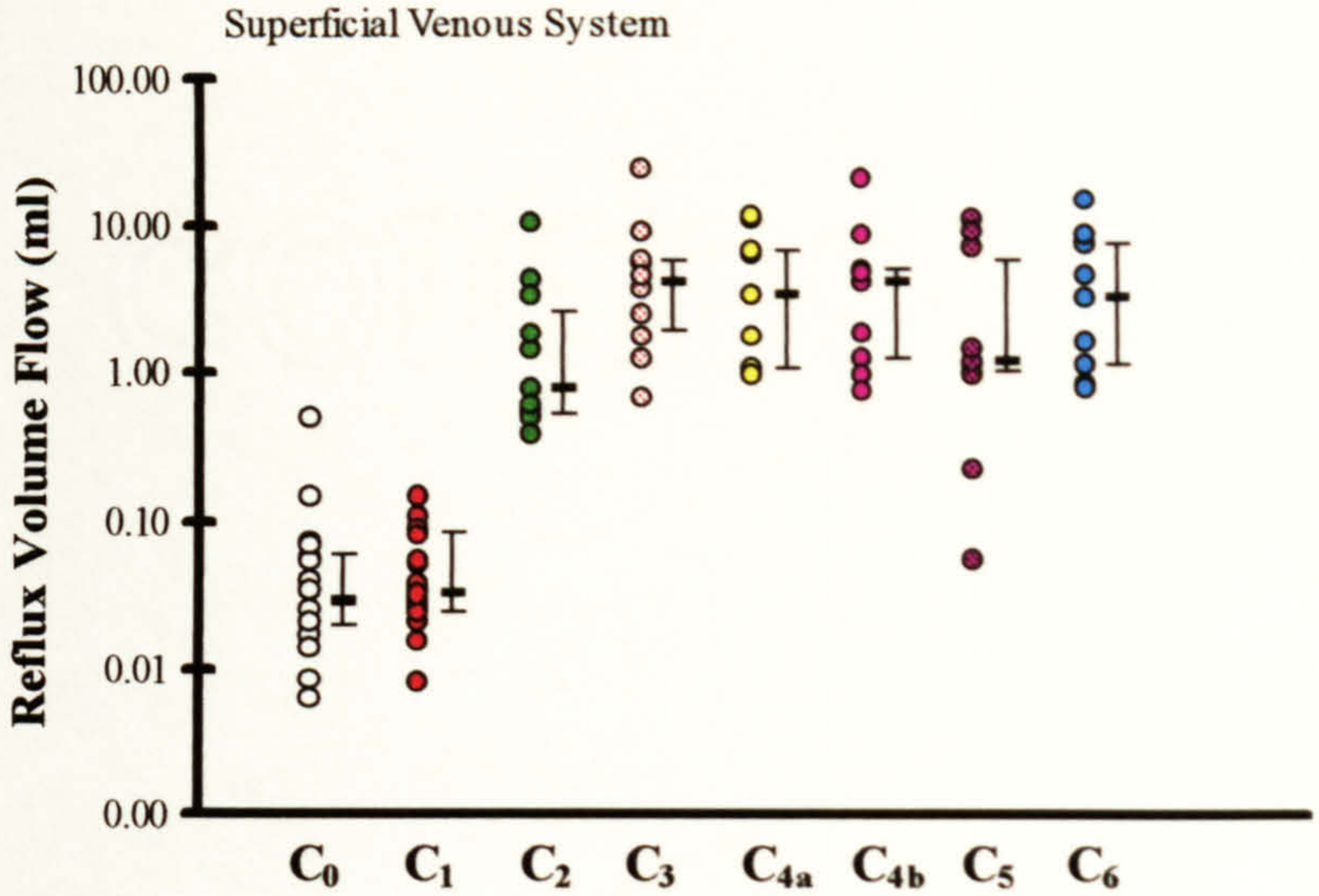
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.06						
C_2	<0.0005	<0.0005					
C_3	<0.0005	<0.0005	0.52				
C_{4a}	<0.0005	<0.0005	0.84	0.56			
C_{4b}	<0.0005	<0.0005	0.84	0.80	0.89		
C_5	<0.0005	<0.0005	0.13	0.04	0.11	0.23	
C_6	<0.0005	<0.0005	0.62	0.77	0.69	0.85	0.05

Figure 3.8.1 Duplex-derived reflux fraction in superficial venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



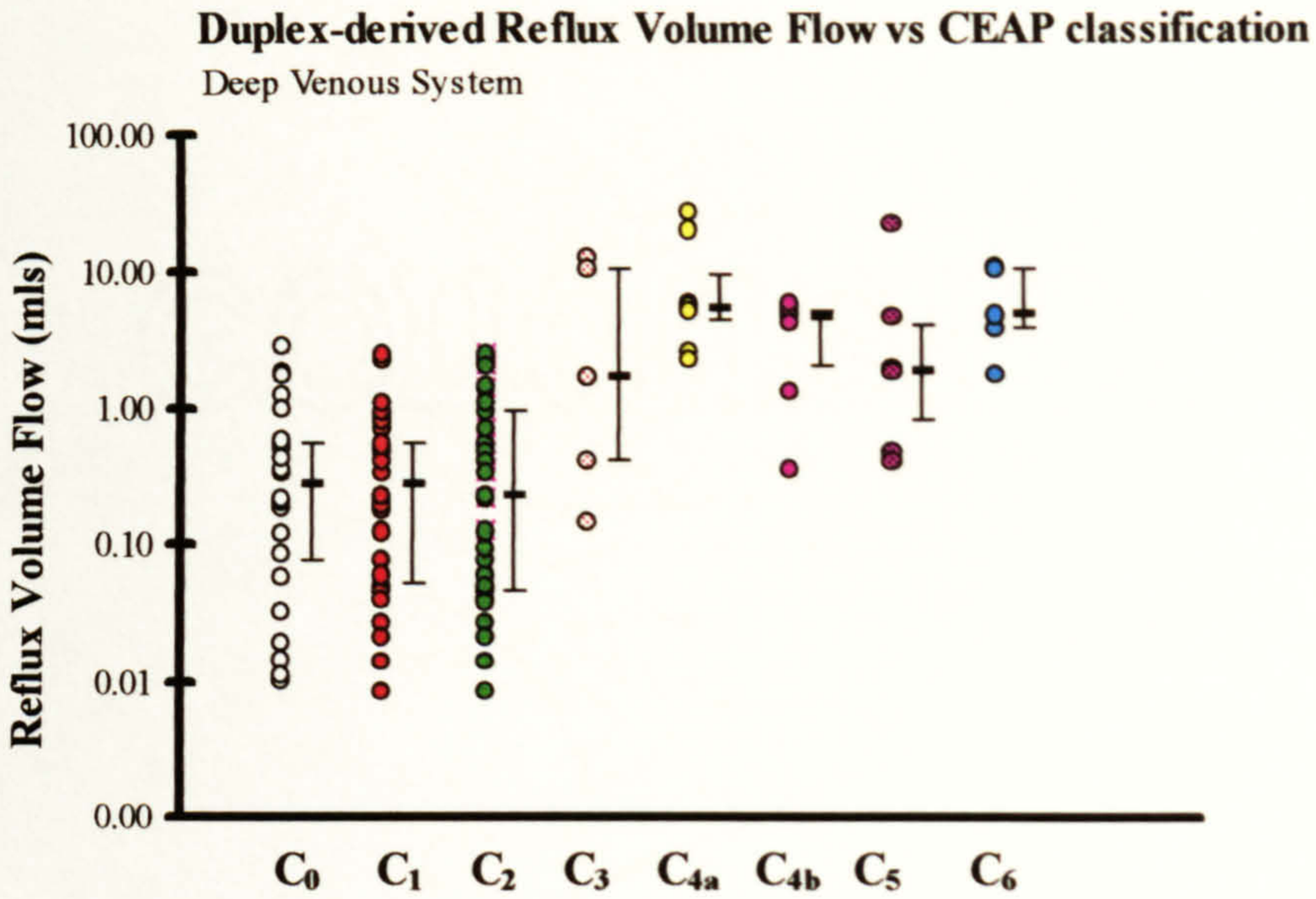
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.89						
C_2	0.30	0.50					
C_3	0.0005	0.0004	0.0002				
C_{4a}	<0.0005	<0.0005	0.0004	0.30			
C_{4b}	0.0002	0.0001	0.0002	0.71	0.05		
C_5	0.0004	0.0002	0.0004	0.36	0.79	0.12	
C_6	0.0006	0.0004	0.0005	0.17	0.50	0.04	0.71

Figure 3.8.2 Duplex-derived reflux fraction in deep venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



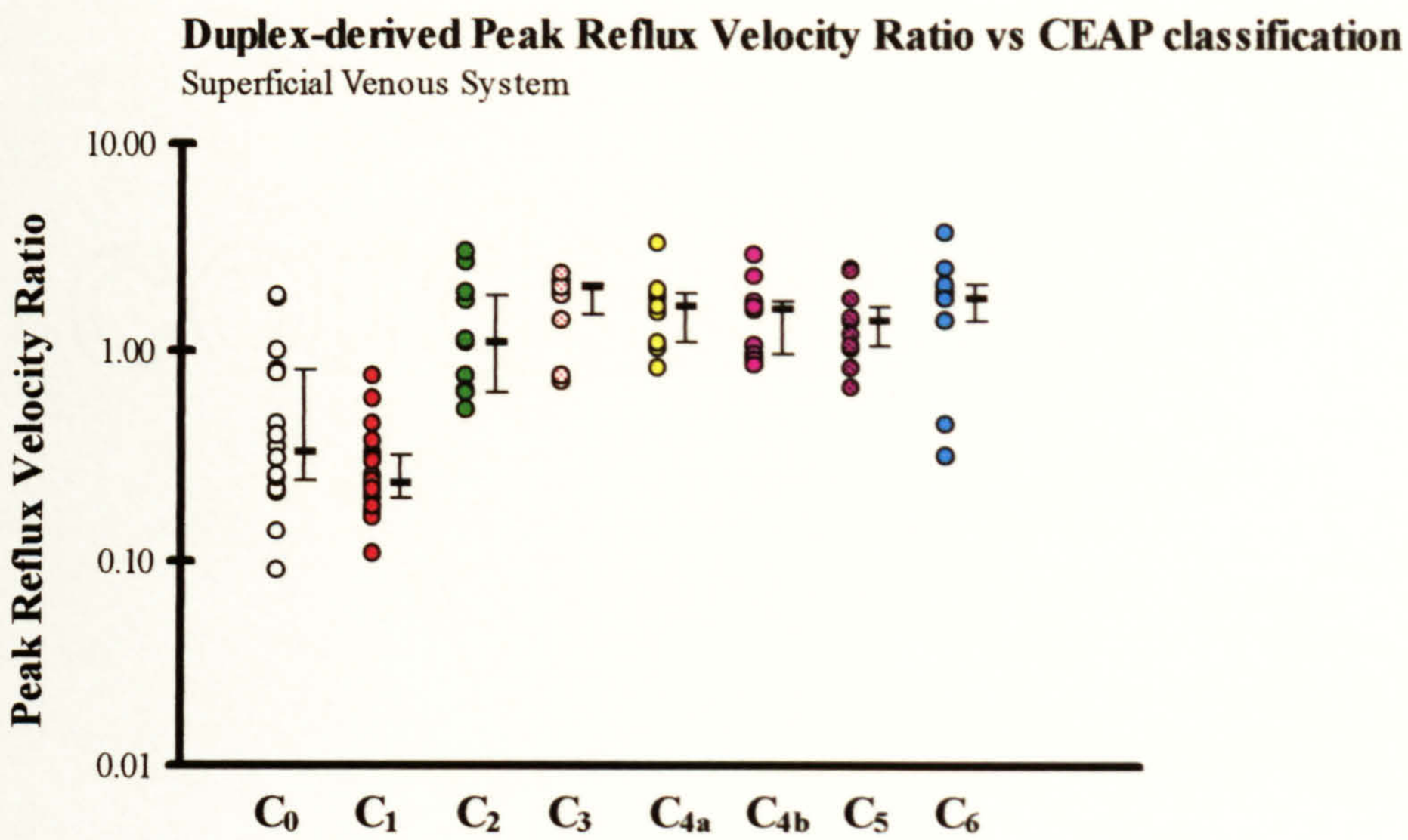
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.35						
C_2	<0.0005	<0.0005					
C_3	<0.0005	<0.0005	0.02				
C_{4a}	<0.0005	<0.0005	0.04	0.87			
C_{4b}	<0.0005	<0.0005	0.05	0.68	0.82		
C_5	0.0004	0.0002	0.49	0.23	0.42	0.47	
C_6	<0.0005	<0.0005	0.06	0.68	0.79	0.85	0.56

Figure 3.8.3 Duplex-derived reflux volume flow in superficial venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



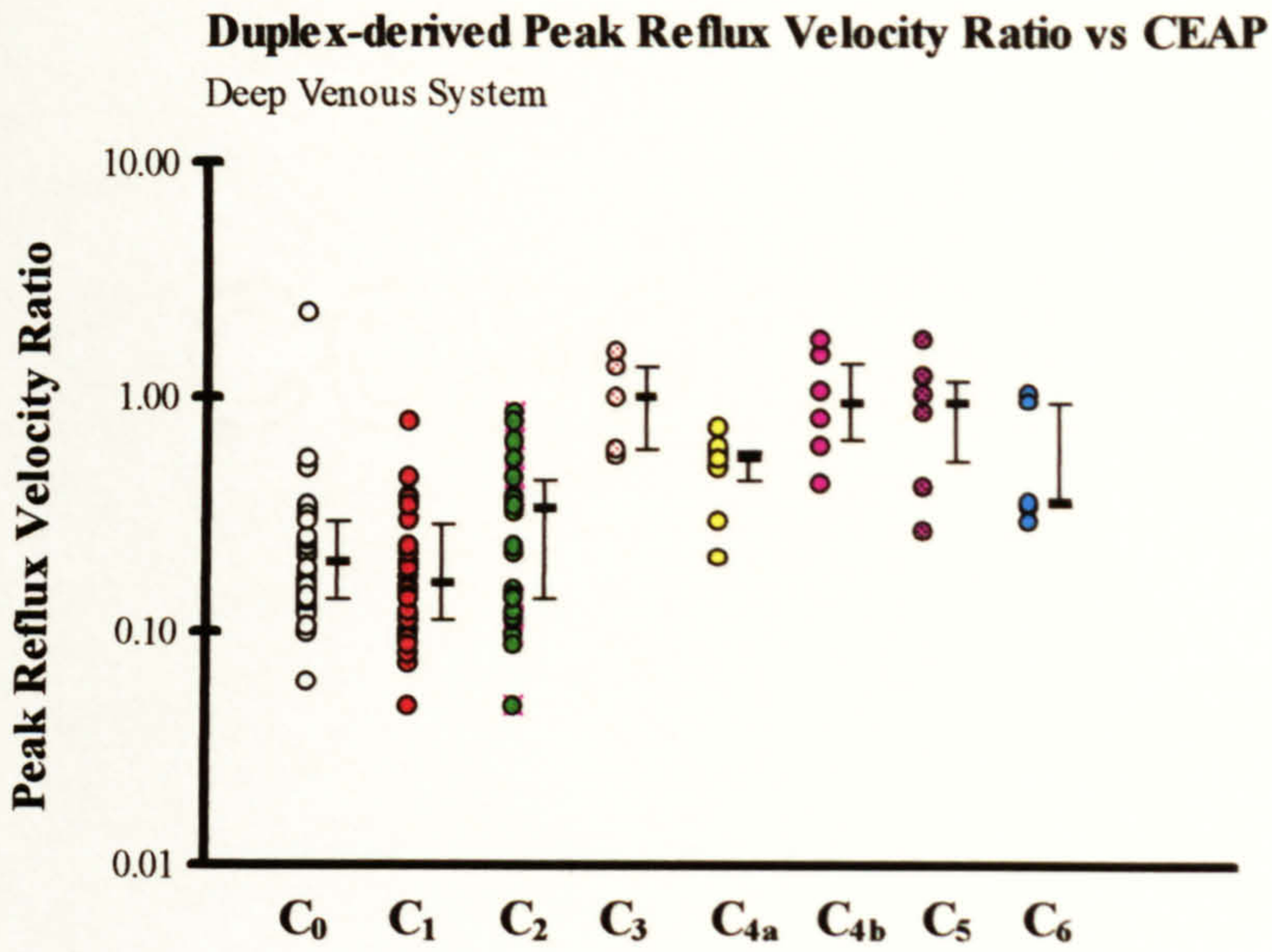
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.90						
C_2	0.27	0.63					
C_3	0.06	0.04	0.06				
C_{4a}	0.0001	<0.0005	0.007	0.24			
C_{4b}	0.002	0.001	0.0006	1.00	0.15		
C_5	0.008	0.007	0.002	0.71	0.05	0.63	
C_6	0.001	0.0006	0.008	0.46	0.55	0.36	0.27

Figure 3.8.4 Duplex-derived reflux volume flow in deep venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



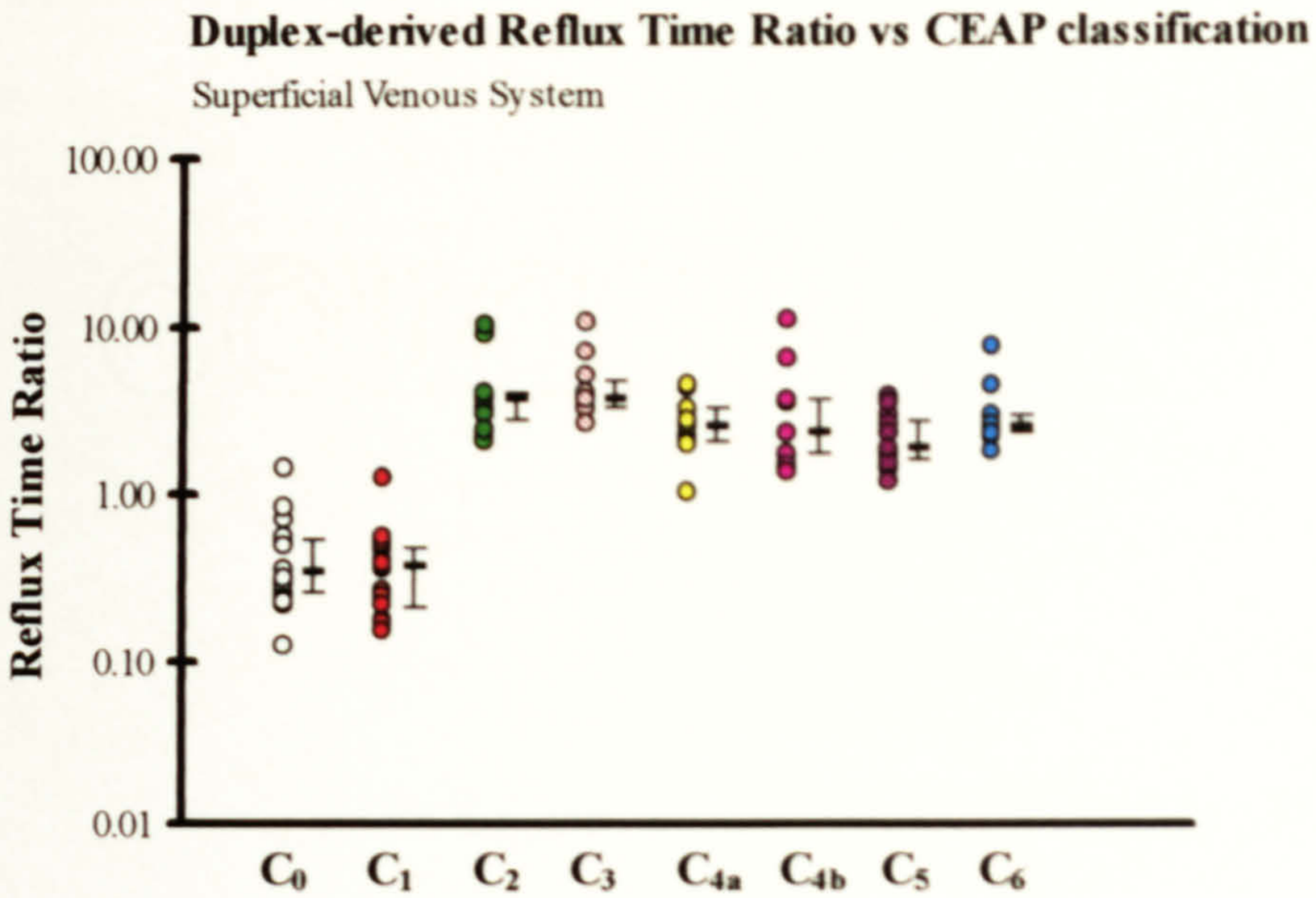
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.07						
C_2	0.004	<0.0005					
C_3	0.0004	<0.0005	0.11				
C_{4a}	0.0008	<0.0005	0.18	0.32			
C_{4b}	0.001	<0.0005	0.38	0.41	0.59		
C_5	0.001	<0.0005	0.41	0.27	0.36	0.79	
C_6	0.002	0.0001	0.42	0.65	0.65	0.45	0.38

Figure 3.8.5 Duplex-derived peak reflux velocity ratio in superficial venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



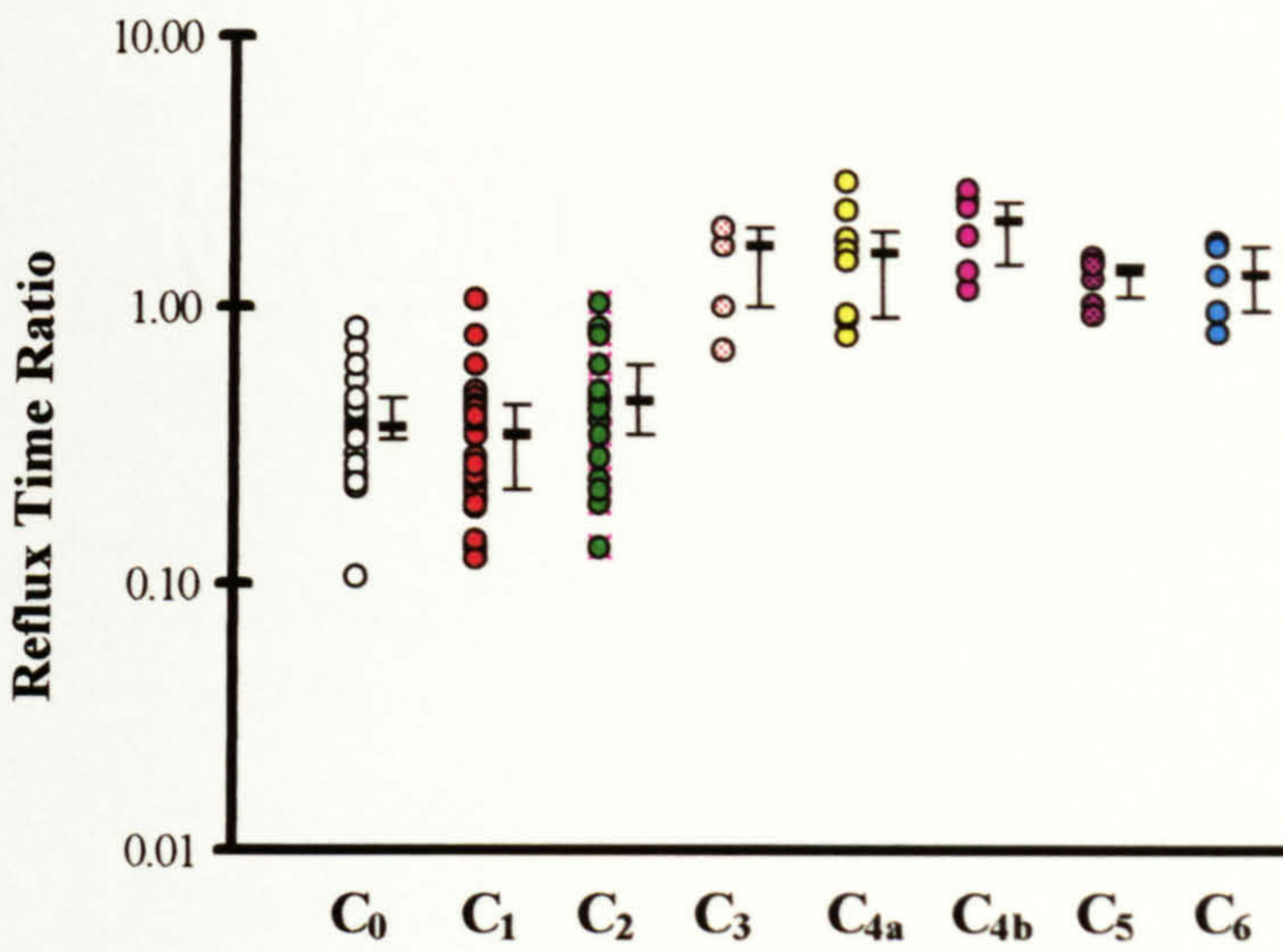
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.33						
C_2	0.36	0.33					
C_3	0.001	0.0006	0.001				
C_{4a}	0.002	0.0007	0.002	0.02			
C_{4b}	0.0009	0.0002	0.0007	0.85	0.03		
C_5	0.002	0.0007	0.0002	0.71	0.15	0.68	
C_6	0.009	0.009	0.009	0.11	0.82	0.10	0.36

Figure 3.8.6 Duplex-derived peak reflux velocity ratio in deep venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



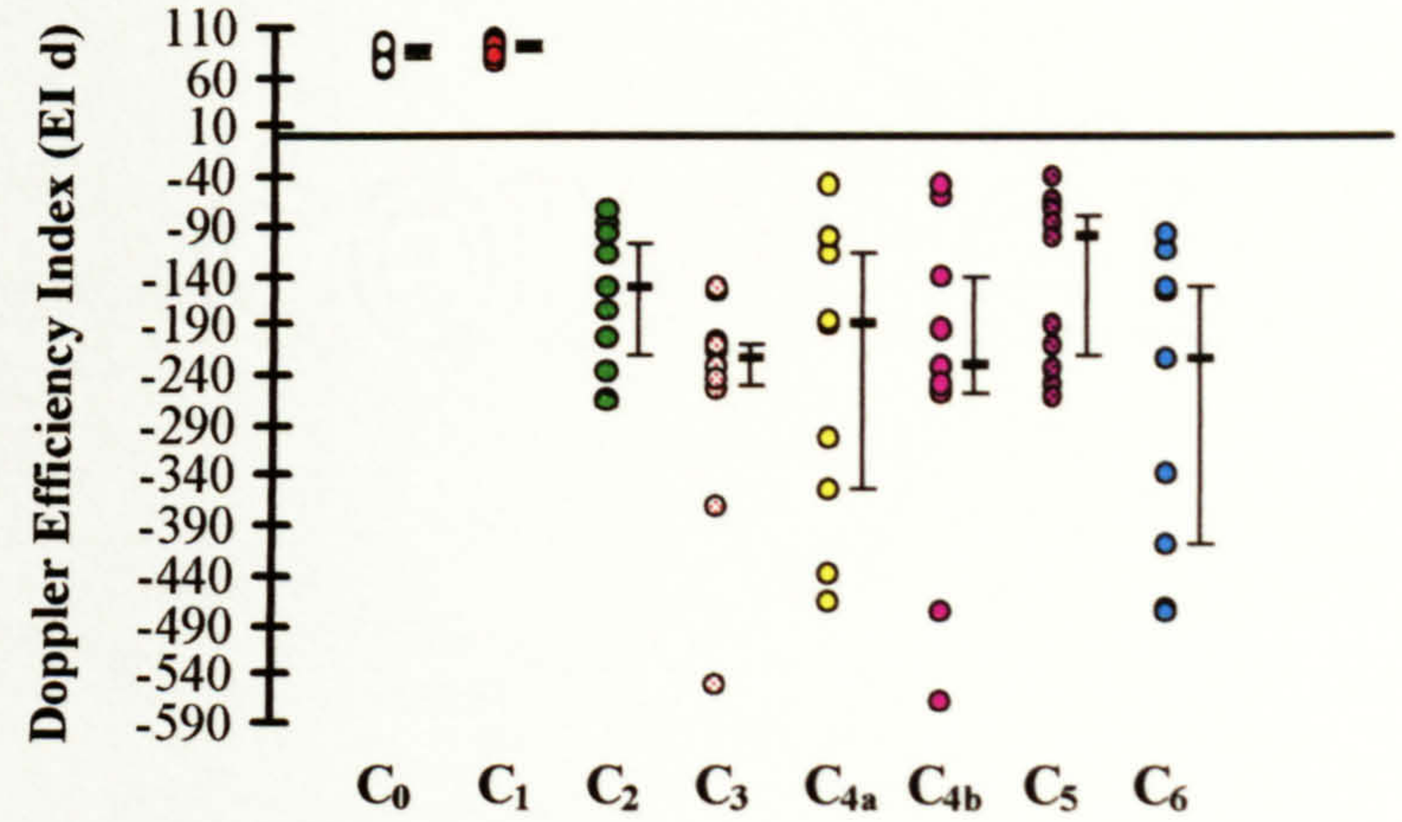
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.52						
C_2	<0.0005	<0.0005					
C_3	<0.0005	<0.0005	0.39				
C_{4a}	0.0001	<0.0005	0.18	0.03			
C_{4b}	0.0001	<0.0005	0.23	0.14	0.82		
C_5	<0.0005	<0.0005	0.007	0.002	0.27	0.38	
C_6	<0.0005	<0.0005	0.22	0.02	0.69	0.75	0.15

Figure 3.8.7 Duplex-derived reflux time ratio in superficial venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



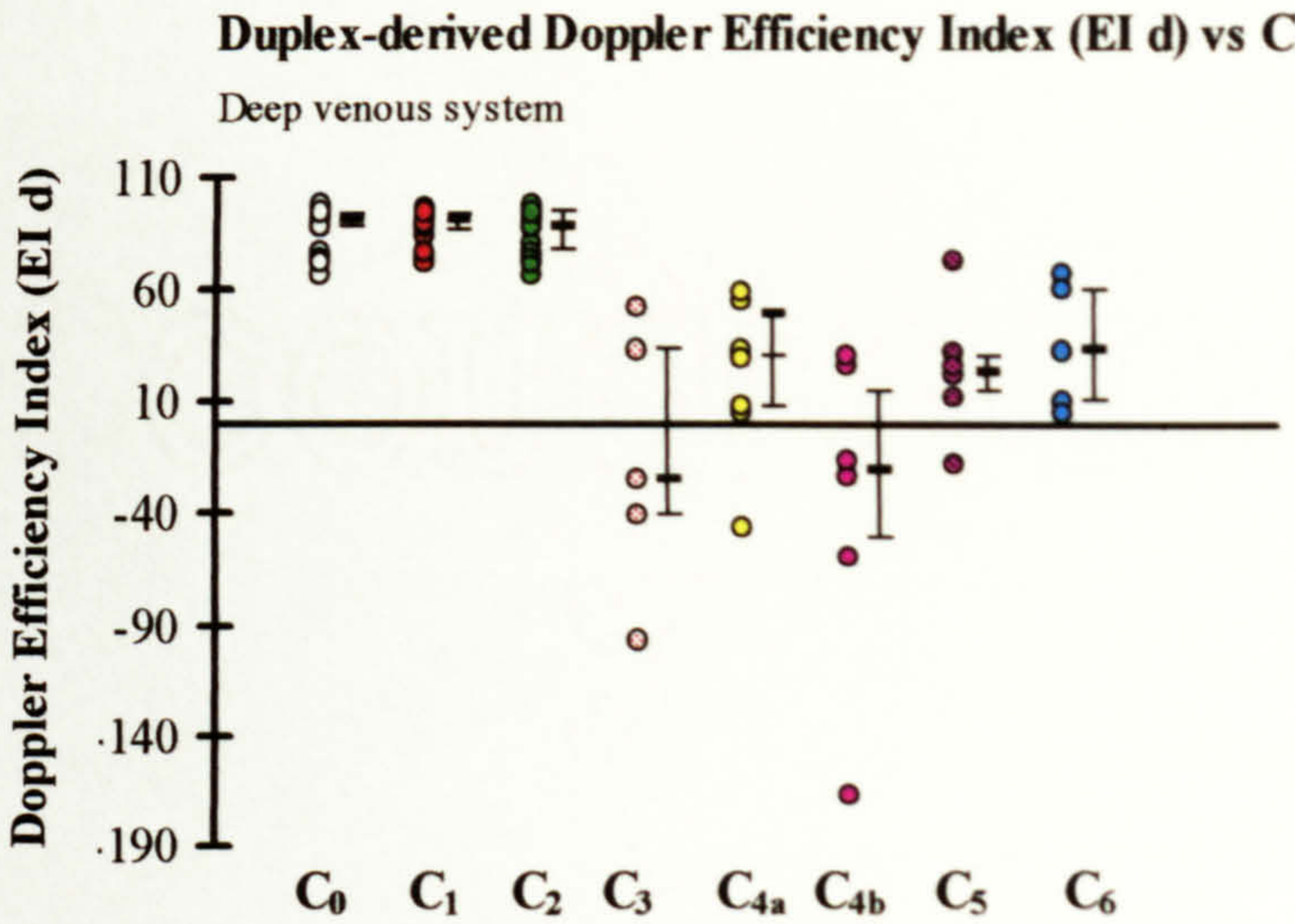
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.23						
C_2	0.24	0.85					
C_3	0.0008	0.0007	0.0002				
C_{4a}	<0.0005	<0.0005	0.0006	1.00			
C_{4b}	0.0002	0.0001	<0.0005	0.20	0.24		
C_5	0.0002	0.0002	0.0002	0.46	0.51	0.07	
C_6	0.0007	0.0006	0.0008	0.52	0.66	0.06	0.85

Figure 3.8.8 Duplex-derived reflux time ratio in deep venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



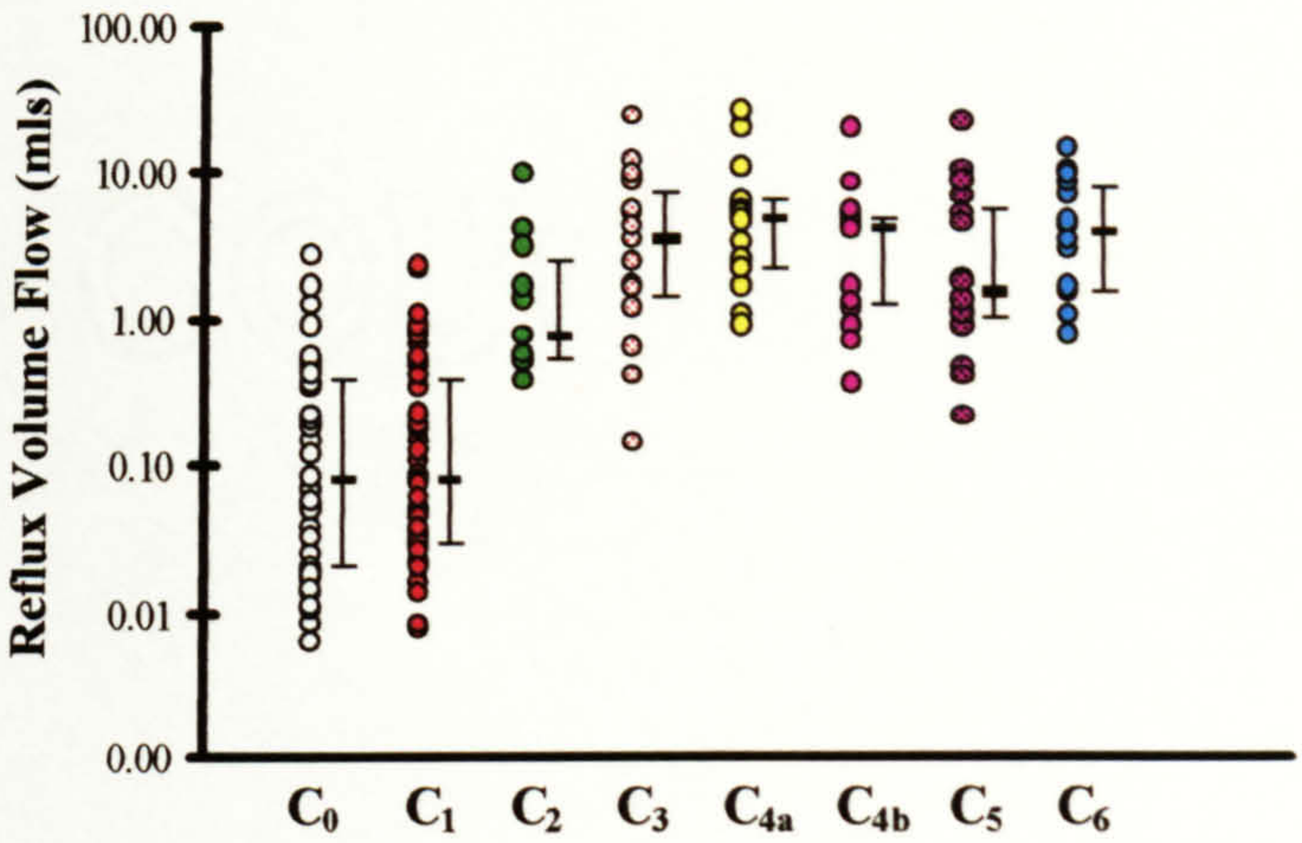
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.06						
C_2	<0.0005	<0.0005					
C_3	<0.0005	<0.0005	0.52				
C_{4a}	<0.0005	<0.0005	0.84	0.56			
C_{4b}	<0.0005	<0.0005	0.84	0.80	0.89		
C_5	<0.0005	<0.0005	0.13	0.04	0.11	0.23	
C_6	<0.0005	<0.0005	0.62	0.77	0.69	0.85	0.05

Figure 3.8.9 Duplex-derived Doppler Efficiency Index in superficial venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



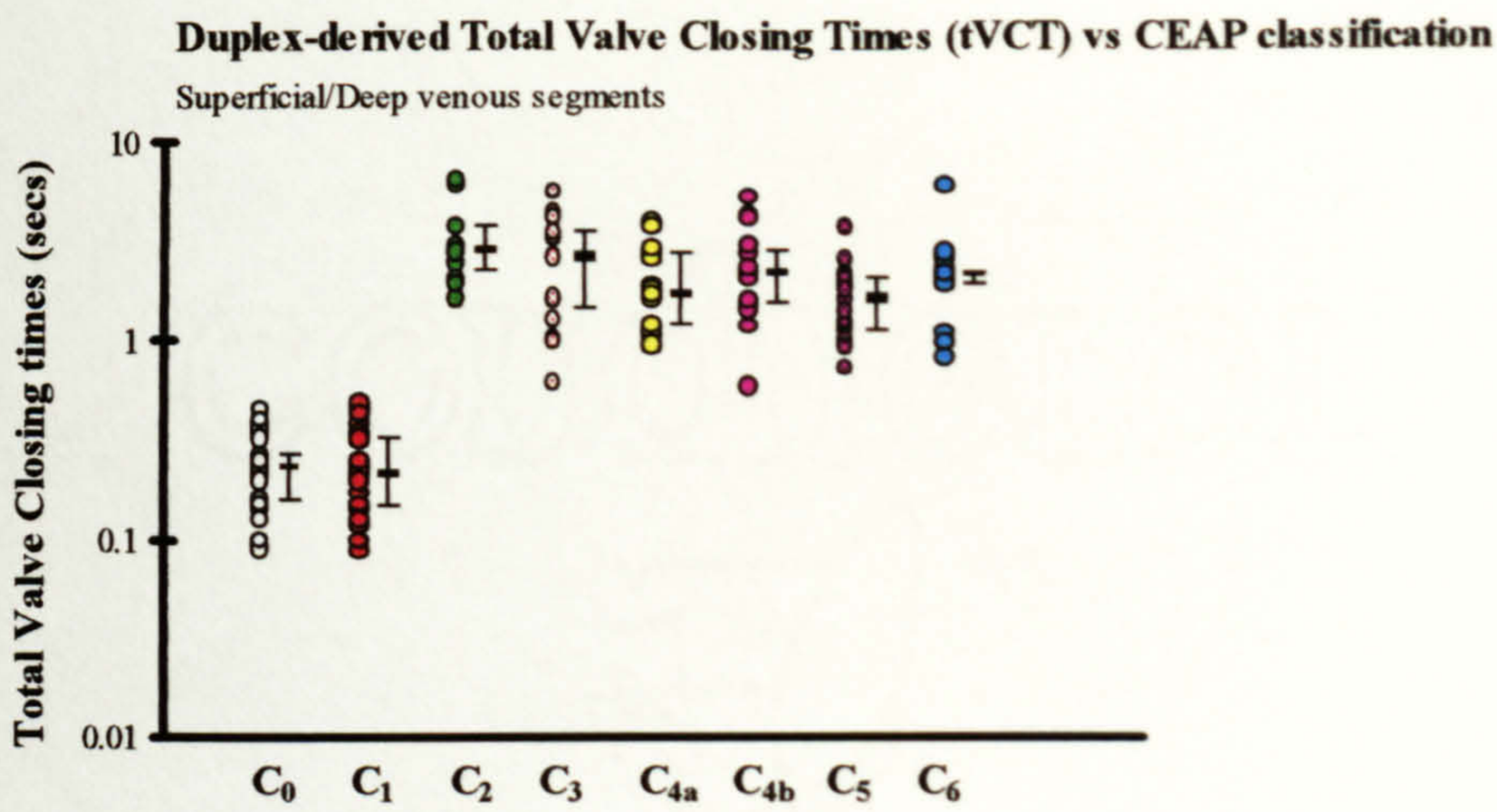
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.89						
C_2	0.71	0.79					
C_3	0.0005	0.0004	0.0002				
C_{4a}	<0.0005	0.0000	0.0004	0.30			
C_{4b}	0.0002	0.0001	0.0005	0.71	0.05		
C_5	0.0004	0.0002	0.0001	0.36	0.79	0.12	
C_6	0.0006	0.0004	0.0002	0.17	0.50	0.04	0.71

Figure 3.8.10 Duplex-derived Doppler efficiency index in deep venous segments in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.78						
C_2	<0.0005	<0.0005					
C_3	<0.0005	<0.0005	0.02				
C_{4a}	<0.0005	<0.0005	0.04	0.67			
C_{4b}	<0.0005	<0.0005	0.05	0.88	0.82		
C_5	<0.0005	<0.0005	0.04	0.53	0.42	0.76	
C_6	<0.0005	<0.0005	0.05	0.77	0.79	0.88	0.63

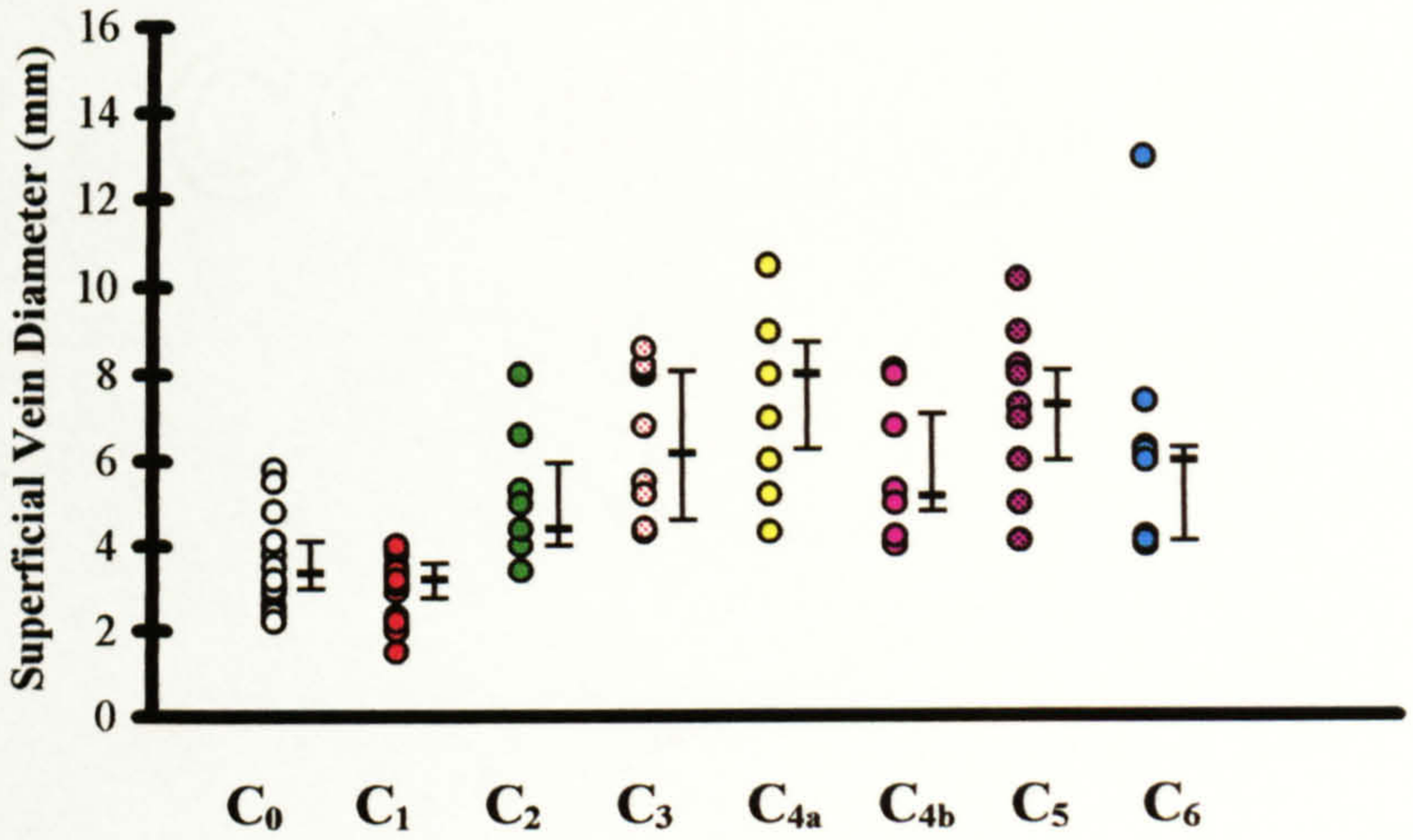
Figure 3.8.11 Duplex-derived total reflux volume flow in corresponding segments in normal subjects with all incompetent segments in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.78						
C_2	<0.0005	<0.0005					
C_3	<0.0005	<0.0005	0.34				
C_{4a}	<0.0005	<0.0005	0.02	0.29			
C_{4b}	<0.0005	<0.0005	0.08	0.59	0.48		
C_5	<0.0005	<0.0005	0.01	0.06	0.38	0.07	
C_6	<0.0005	<0.0005	0.04	0.31	0.51	0.66	0.07

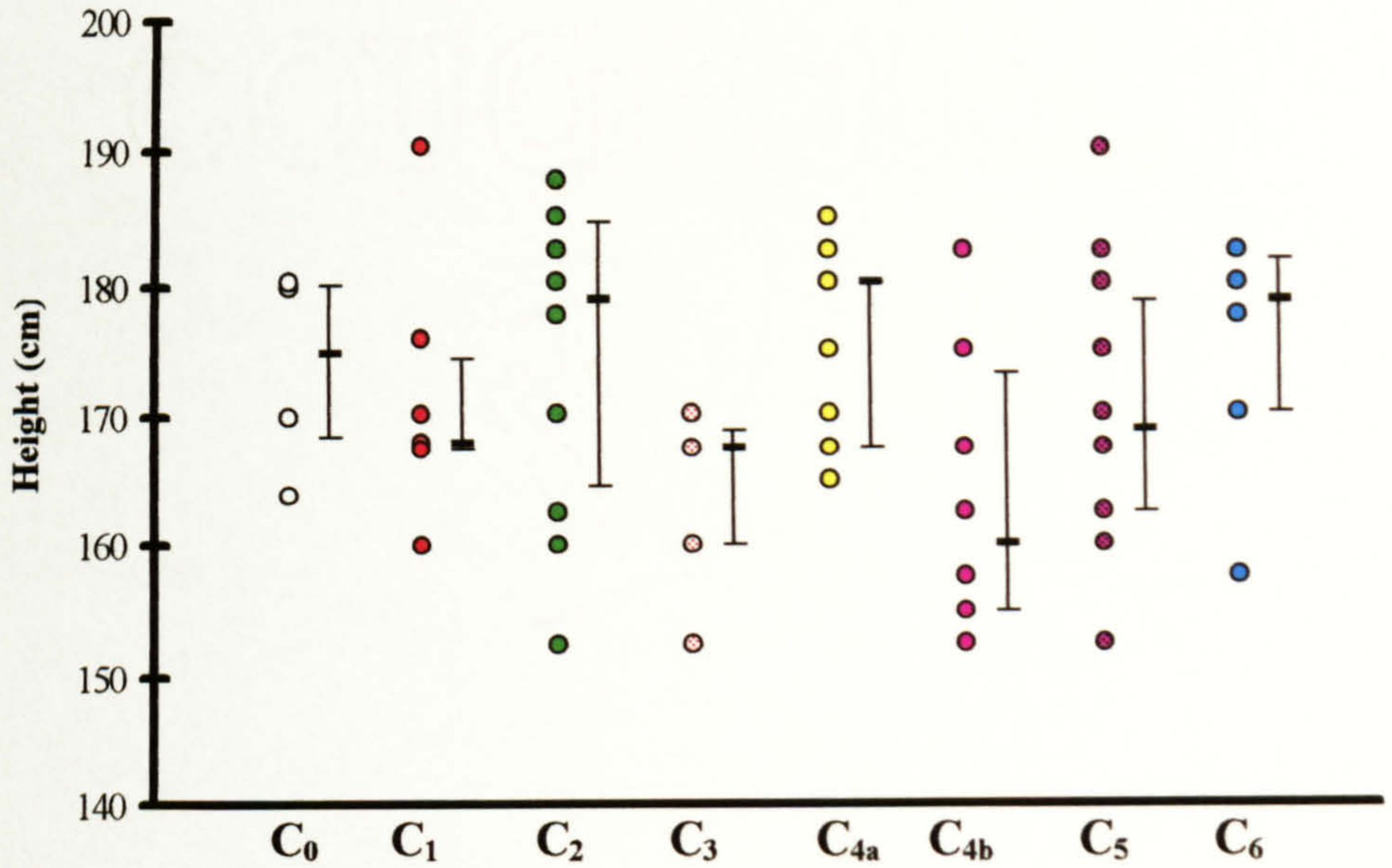
Figure 3.8.12 Duplex-derived total valve closing times (tVCT) in corresponding segments in normal subjects with all incompetent segments in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.

Duplex-derived superficial vein diameters vs CEAP classification



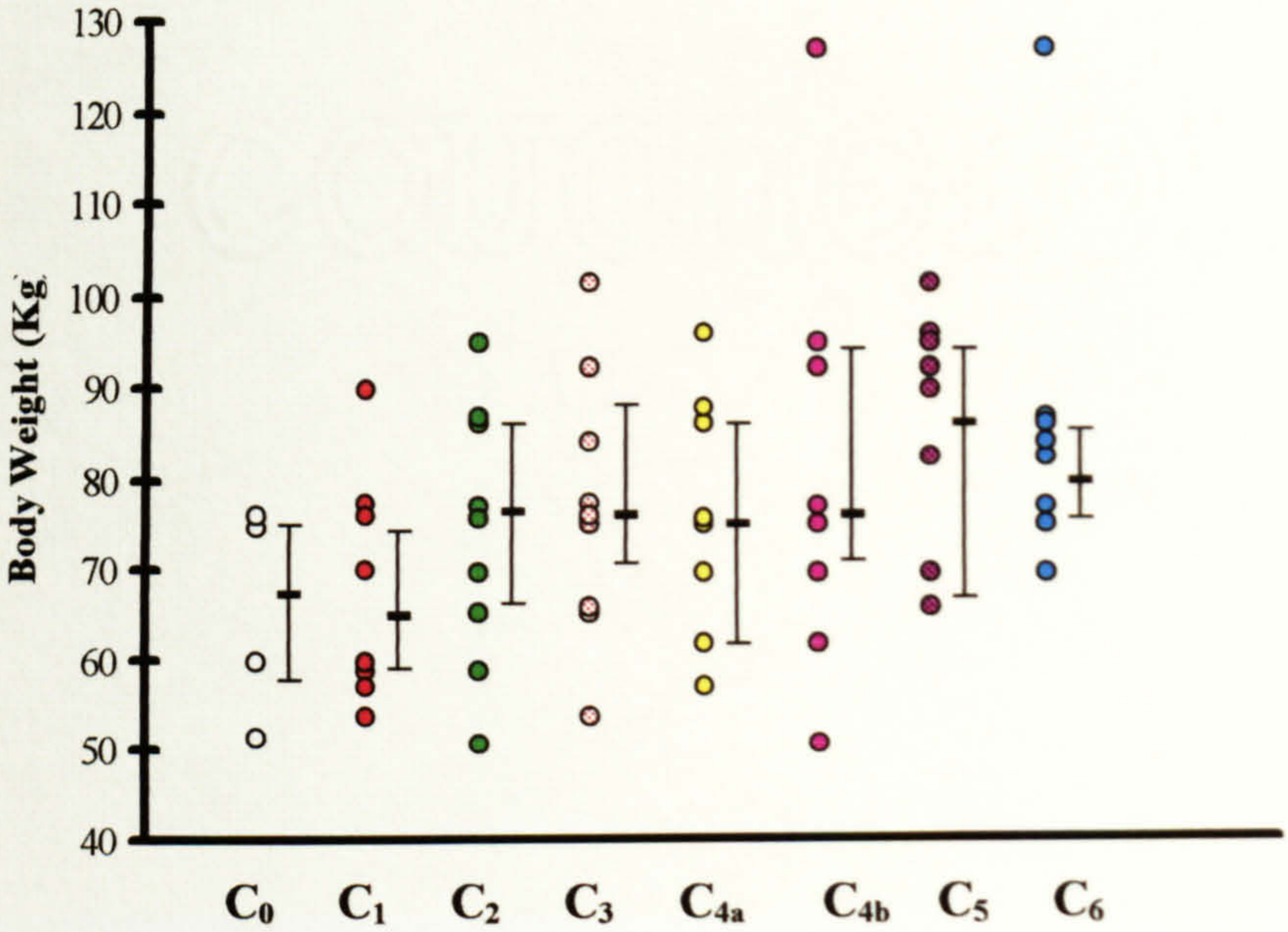
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.29						
C_2	0.006	0.0001					
C_3	0.0004	<0.0005	0.06				
C_{4a}	0.0001	<0.0005	0.01	0.27			
C_{4b}	0.002	0.0001	0.27	0.34	0.06		
C_5	0.0001	<0.0005	0.01	0.41	0.64	0.11	
C_6	0.001	<0.0005	0.56	0.25	0.06	0.73	0.12

Figure 3.8.13 Duplex-derived superficial vein diameters in normal subjects and in patients classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



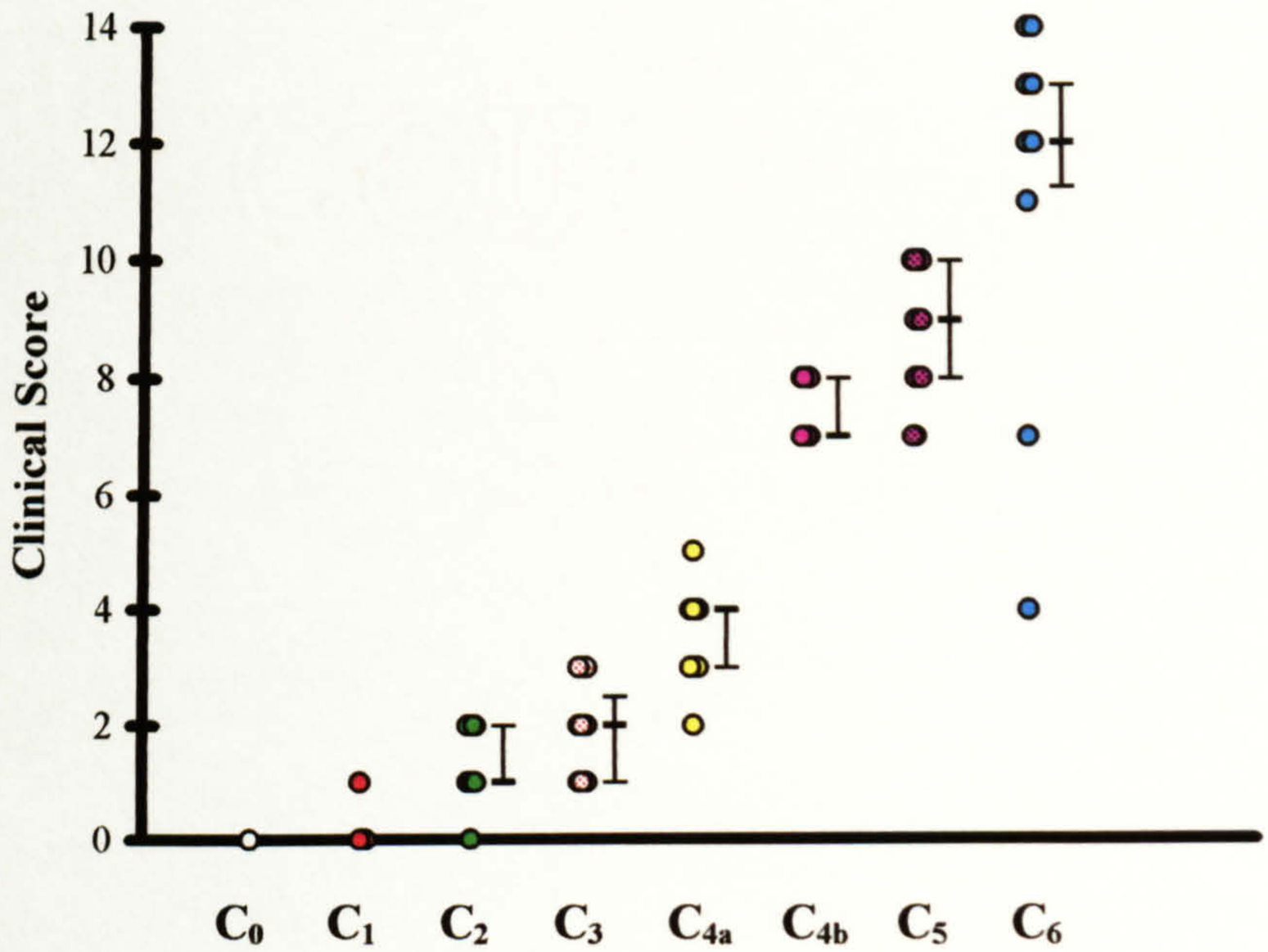
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.37						
C_2	0.53	0.36					
C_3	0.03	0.05	0.03				
C_{4a}	0.24	0.24	0.85	0.004			
C_{4b}	0.04	0.07	0.04	0.85	0.01		
C_5	0.53	0.76	0.42	0.16	0.21	0.19	
C_6	0.20	0.07	0.78	0.003	0.82	0.01	0.25

Figure 3.8.14 Variations in subject heights (cm) in normal subjects and in patients classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



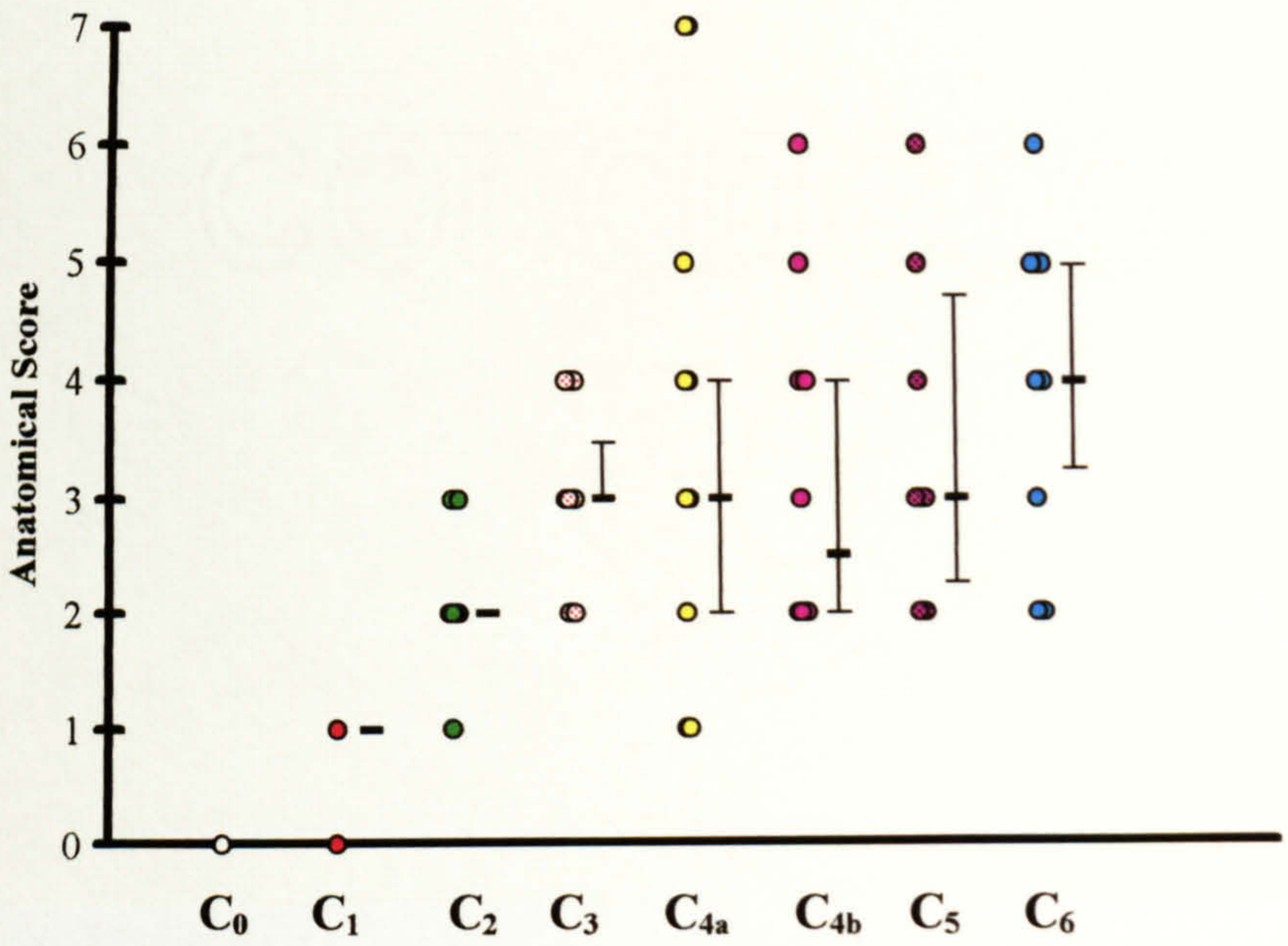
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.78						
C_2	0.15	0.27					
C_3	0.03	0.08	0.75				
C_{4a}	0.24	0.43	0.73	0.39			
C_{4b}	0.07	0.09	0.56	0.83	0.33		
C_5	0.03	0.03	0.22	0.47	0.16	0.81	
C_6	0.004	0.01	0.44	0.50	0.16	0.70	0.93

Figure 3.8.15 Variations in subject body weight (kg) in normal subjects and in patients classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



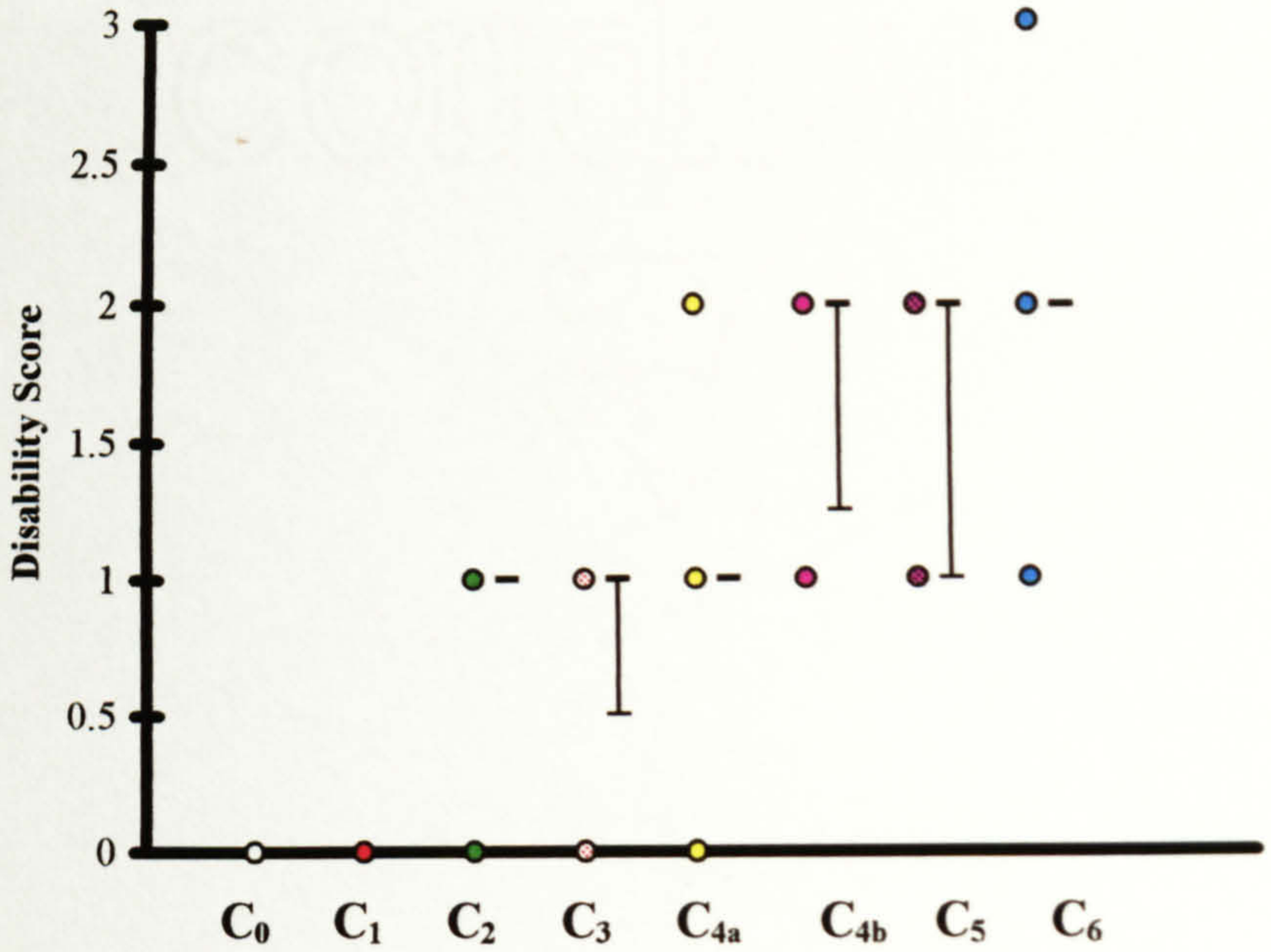
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.37						
C_2	0.0005	0.0005					
C_3	0.0001	0.0001	0.11				
C_{4a}	0.0001	<0.0005	0.0001	0.0004			
C_{4b}	0.0001	<0.0005	0.0001	0.0001	<0.0005		
C_5	0.0002	0.0001	0.0001	0.0001	<0.0005	0.006	
C_6	0.0002	0.0001	0.0001	0.0001	<0.0005	0.0001	0.0002

Figure 3.8.16 CEAP method of clinical scoring in normal subjects and in patients classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



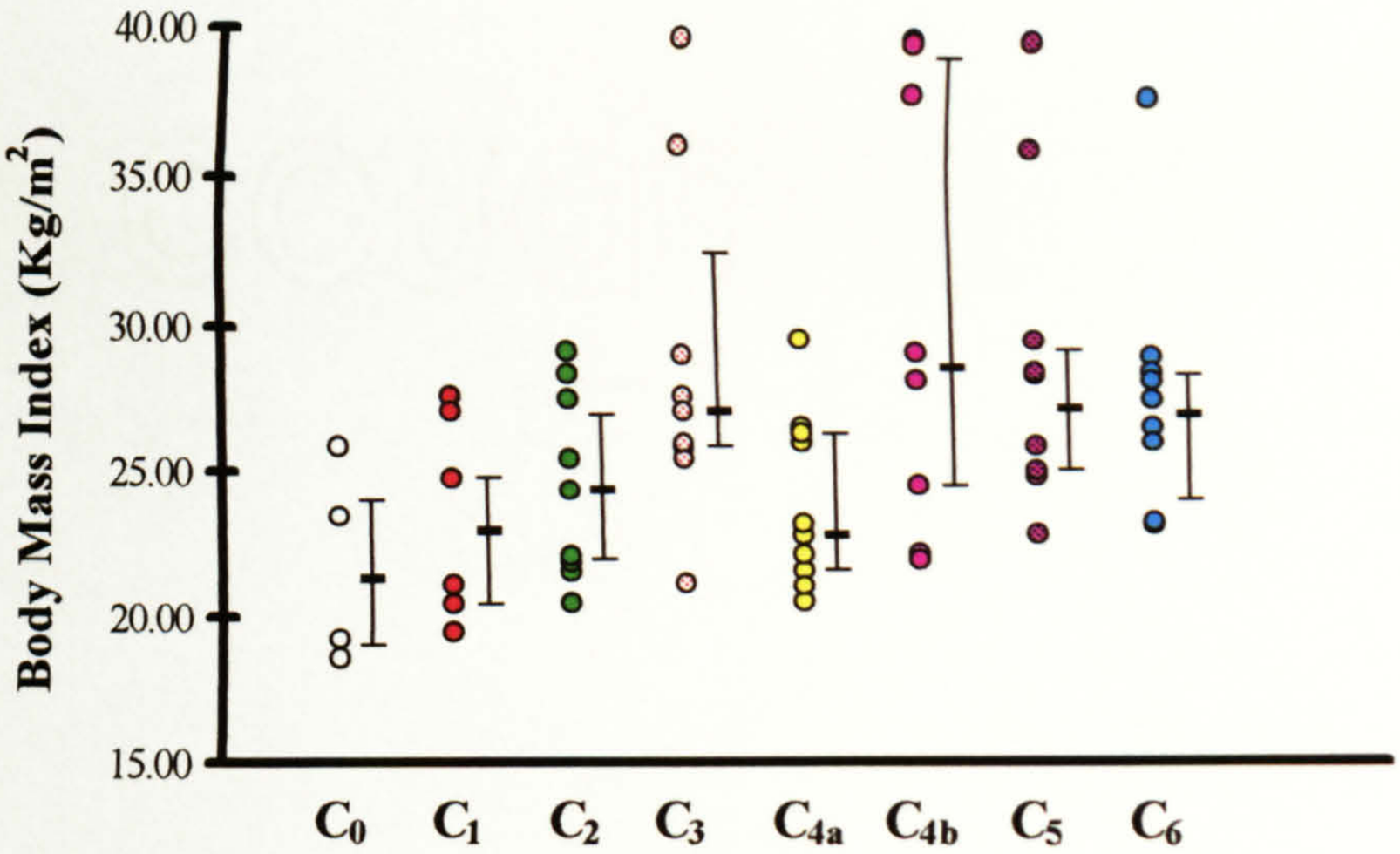
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.0002						
C_2	0.0001	0.0002					
C_3	0.0001	<0.0005	0.004				
C_{4a}	0.0001	0.0006	0.07	0.65			
C_{4b}	0.0002	0.0001	0.06	0.76	0.65		
C_5	0.0002	0.0001	0.01	0.68	0.92	0.43	
C_6	0.0002	0.0001	0.003	0.17	0.65	0.24	0.67

Figure 3.8.17 CEAP method of anatomical scoring in normal subjects and in patients classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



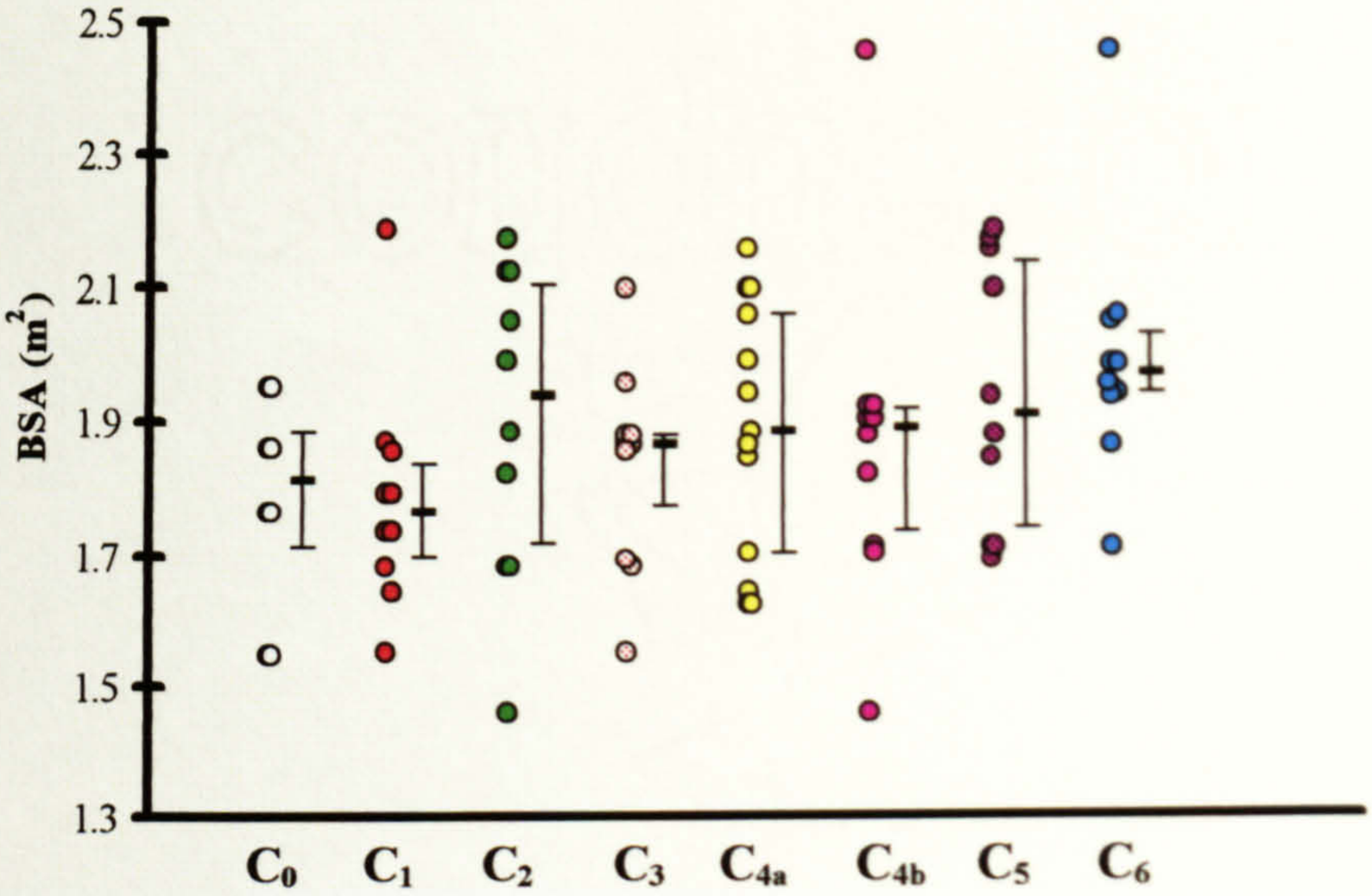
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	1.00						
C_2	0.001	0.0004					
C_3	0.002	0.0008	0.70				
C_{4a}	0.0006	0.0002	0.10	0.06			
C_{4b}	0.0001	<0.0005	0.001	0.0008	0.10		
C_5	0.0001	<0.0005	0.003	0.002	0.21	0.64	
C_6	0.0001	<0.0005	0.0005	0.0003	0.03	0.42	0.24

Figure 3.8.18 CEAP method of disability scoring in normal subjects and in patients classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.21						
C_2	0.10	0.24					
C_3	0.002	0.005	0.05				
C_{4a}	0.14	0.43	0.61	0.02			
C_{4b}	0.01	0.02	0.04	0.94	0.02		
C_5	0.01	0.007	0.04	0.57	0.02	0.96	
C_6	0.01	0.02	0.14	0.64	0.01	0.40	0.73

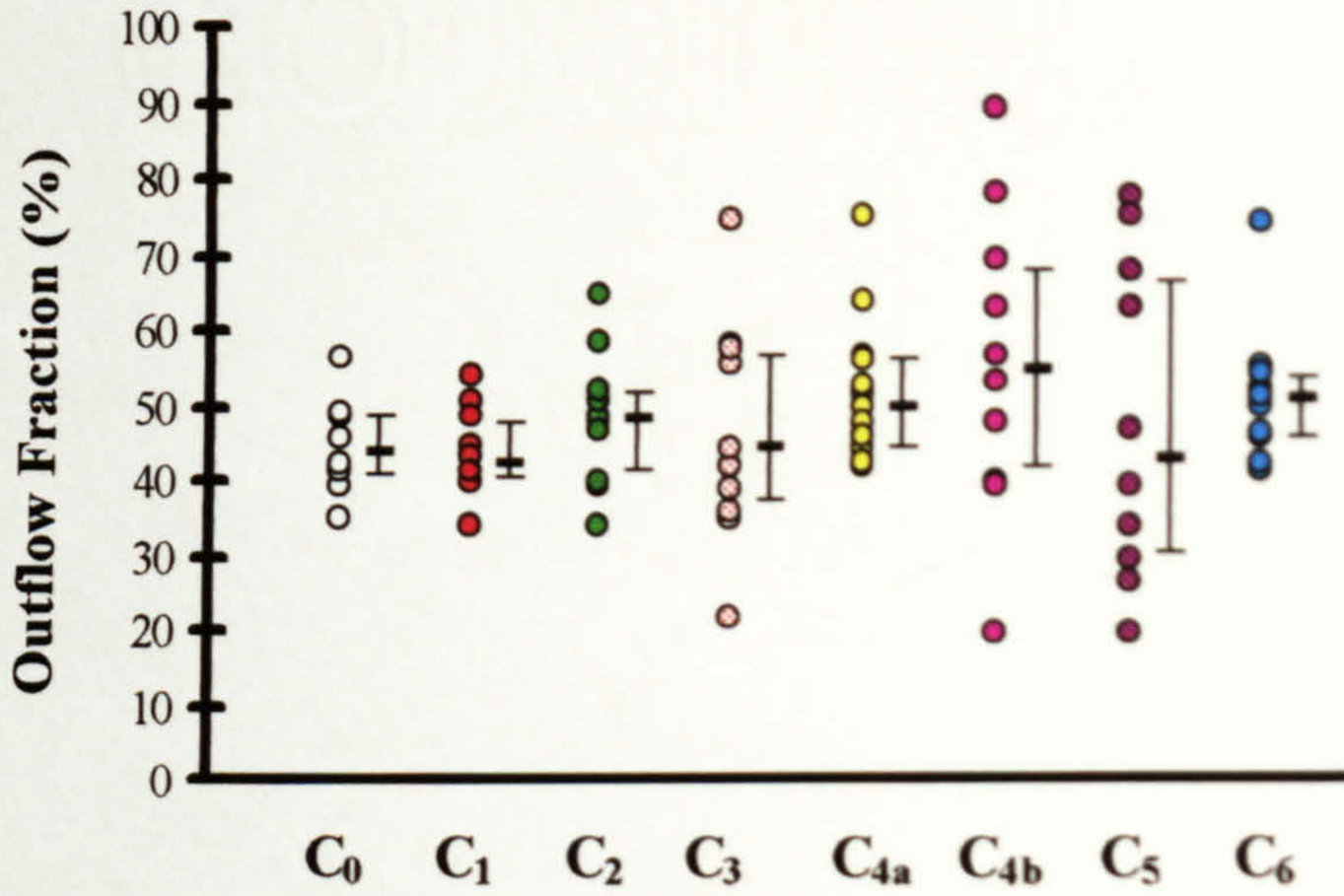
Figure 3.8.19 Variations in the body mass index in normal subjects and in patients classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.72						
C_2	0.21	0.24					
C_3	0.32	0.19	0.34				
C_{4a}	0.19	0.22	0.70	0.43			
C_{4b}	0.59	0.17	0.64	0.52	0.68		
C_5	0.21	0.12	0.57	0.23	0.45	0.47	
C_6	0.01	0.01	0.67	0.02	0.36	0.02	0.56

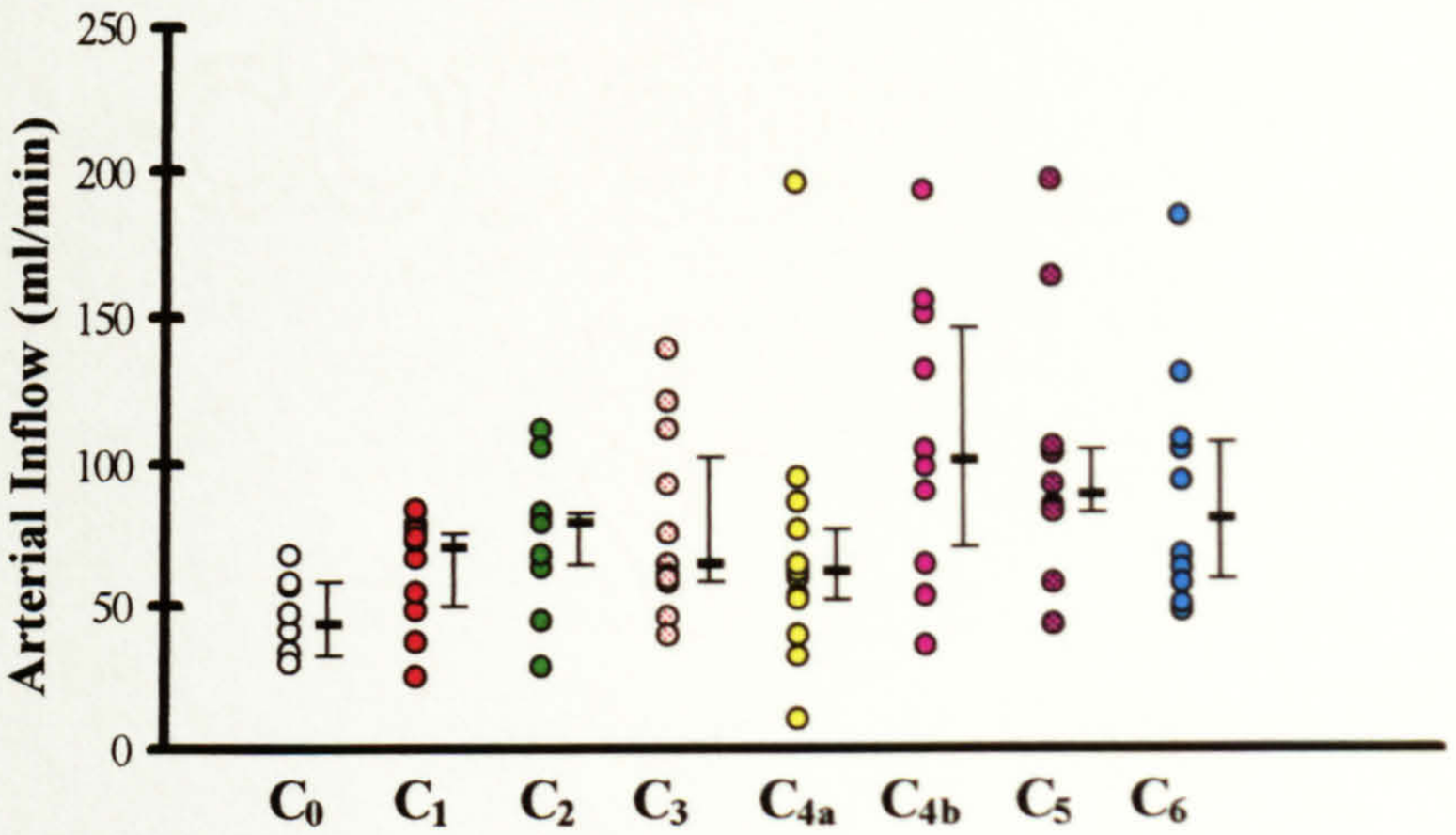
Figure 3.8.20 Variations in the body surface area in normal subjects and in patients classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.

Air plethysmography Results



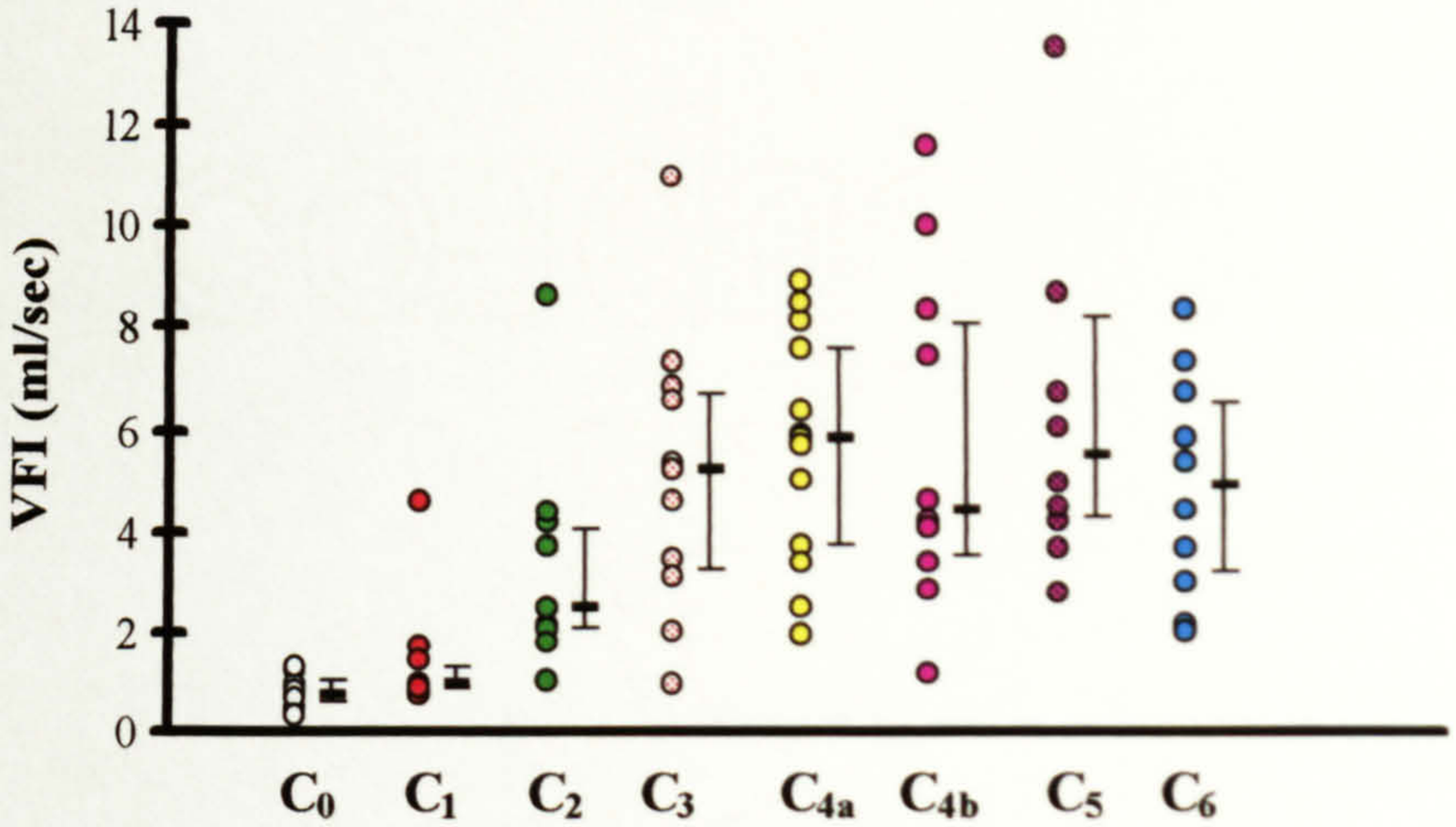
APG-Derived Outflow Fraction 2-Tailed P values Corrected for ties (Mann-Whitney U Test)							
CEAP classification	C_0	C_1	C_2	C_3	C_{4a}	C_{4b}	C_5
C_1	0.65						
C_2	0.37	0.34					
C_3	1.00	0.57	0.52				
C_{4a}	0.11	0.02	0.53	0.25			
C_{4b}	0.18	0.13	0.36	0.23	0.57		
C_5	0.92	0.96	0.70	0.94	0.45	0.36	
C_6	0.09	0.02	0.54	0.36	0.95	0.54	0.54

Figure 3.8.21 APG-derived Outflow Fraction (%) in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



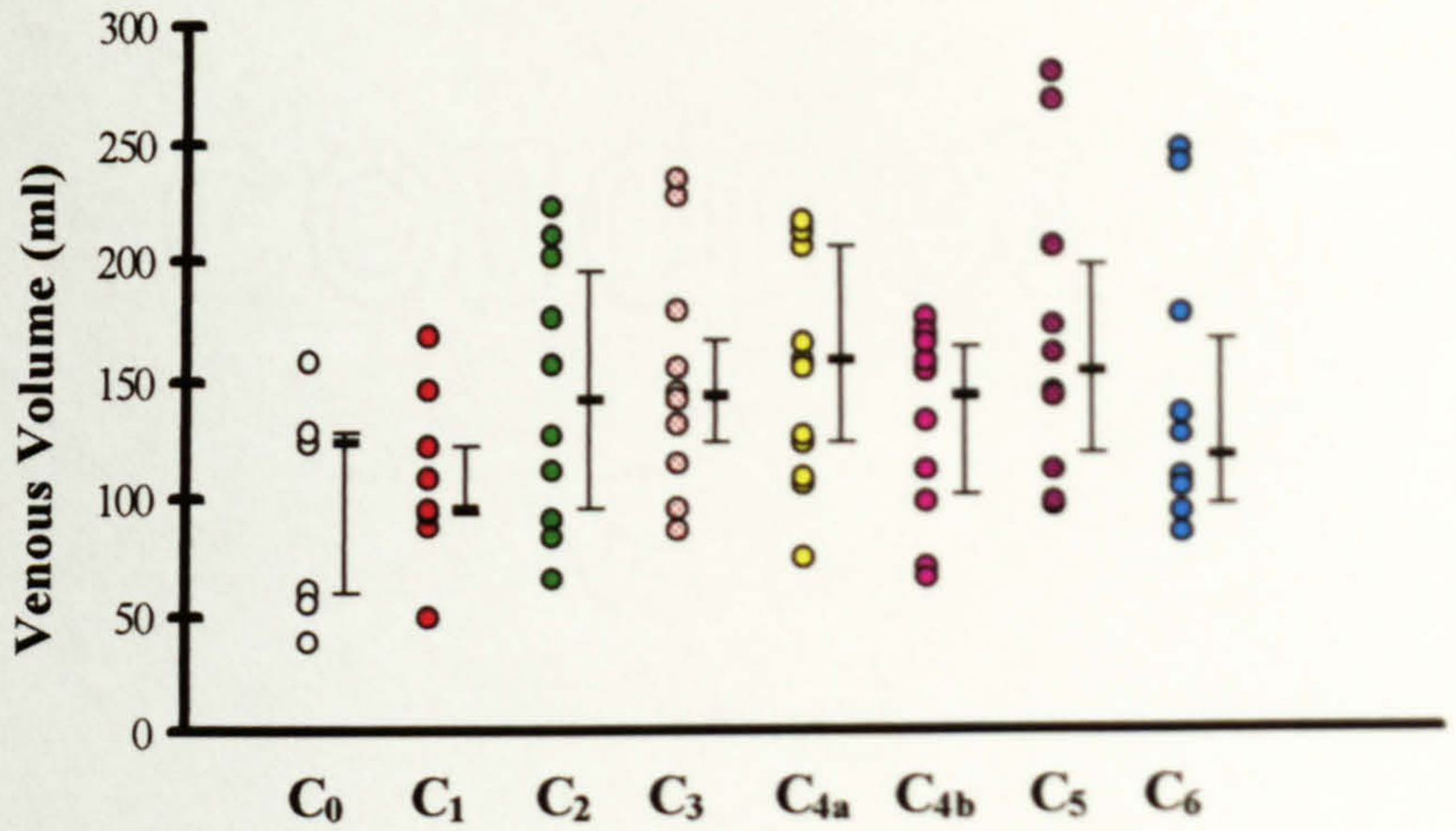
APG-Derived Arterial Inflow 2-Tailed P values Corrected for ties (Mann-Whitney U Test)							
CEAP classification	**C_0**	**C_1**	**C_2**	**C_3**	**C_{4a}**	**C_{4b}**	**C_5**
C_1	0.07						
C_2	0.02	0.19					
C_3	0.01	0.39	0.88				
C_{4a}	0.09	0.90	0.23	0.38			
C_{4b}	0.005	0.03	0.13	0.20	0.04		
C_5	0.002	0.008	0.09	0.32	0.04	0.76	
C_6	0.007	0.15	0.59	0.62	0.18	0.42	0.70

Figure 3.8.22 APG-derived Arterial Inflow (ml/min) in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



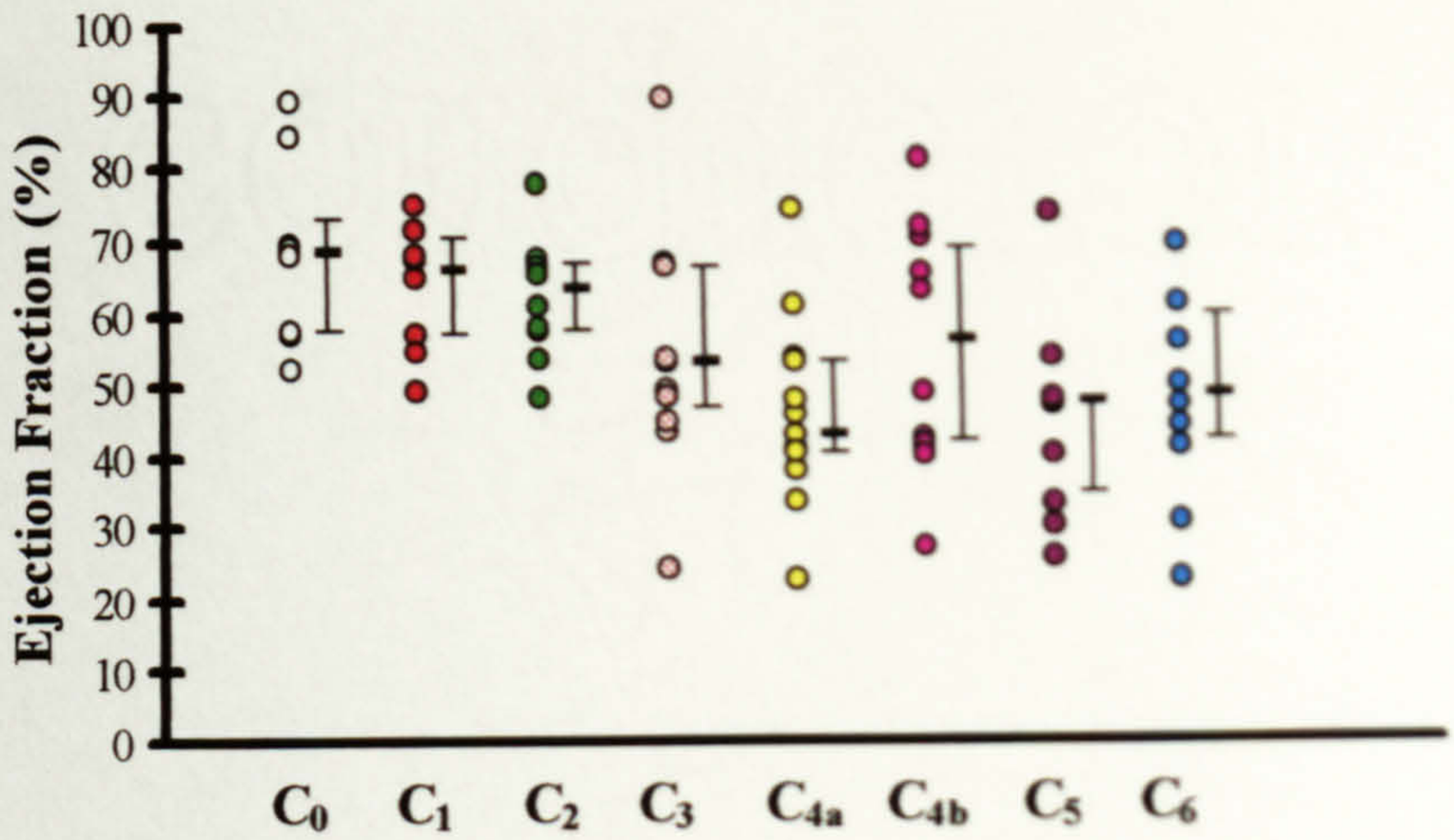
APG-Derived Venous Filling Index 2-Tailed P values Corrected for ties (Mann-Whitney U Test)							
CEAP classification	**C_0**	**C_1**	**C_2**	**C_3**	**C_{4a}**	**C_{4b}**	**C_5**
C_1	0.07						
C_2	0.0007	0.003					
C_3	0.0005	0.001	0.10				
C_{4a}	0.0002	0.0002	0.02	0.50			
C_{4b}	0.0007	0.001	0.06	0.72	0.90		
C_5	0.0004	0.0005	0.008	0.48	0.70	0.54	
C_6	0.0004	0.0007	0.11	0.94	0.42	0.65	0.25

Figure 3.8.23 APG-derived Venous Filling Index (ml/sec) in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



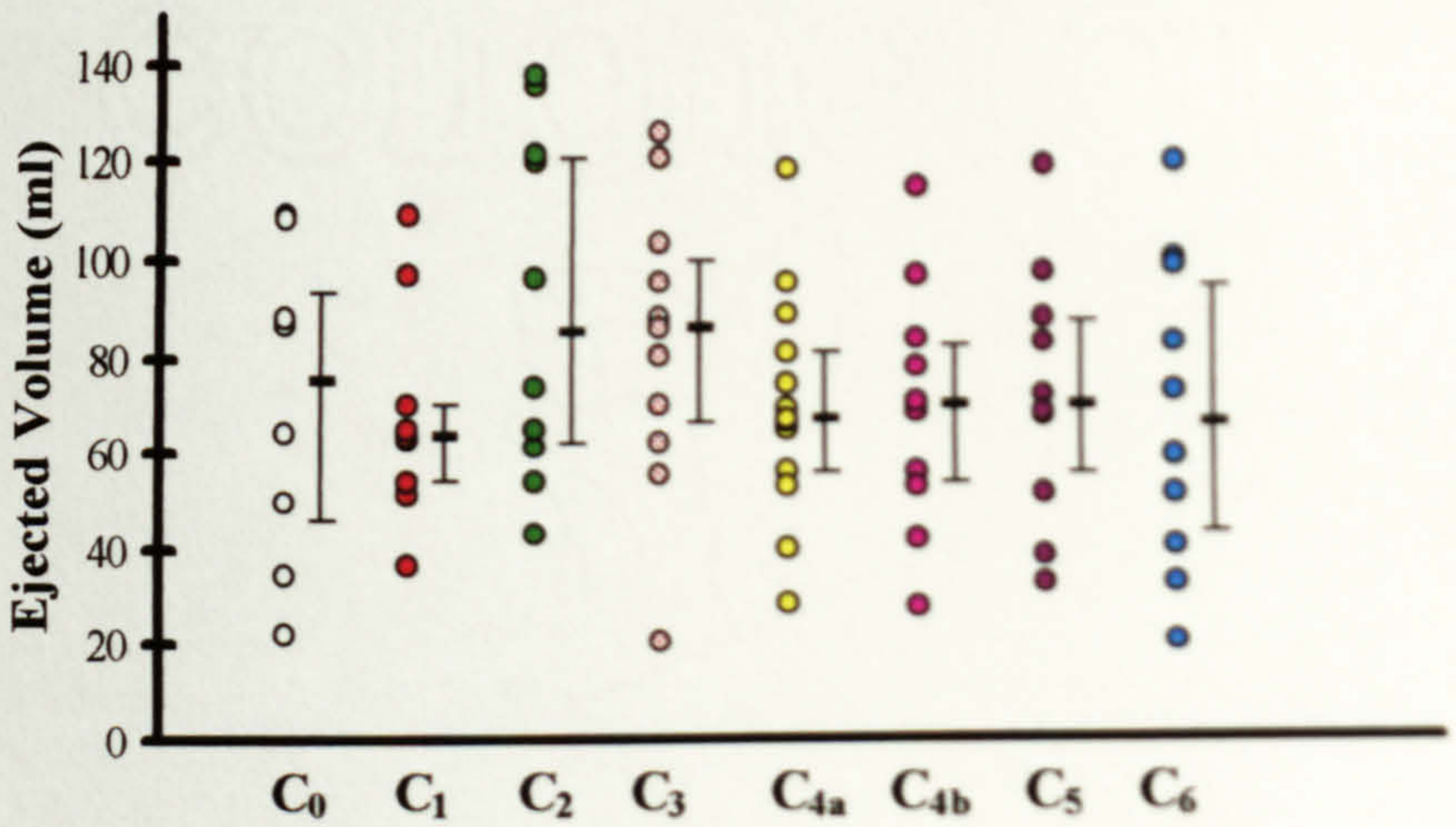
APG-Derived Venous Volume 2-Tailed P values Corrected for ties (Mann-Whitney U Test)							
CEAP classification	**C_0**	**C_1**	**C_2**	**C_3**	**C_{4a}**	**C_{4b}**	**C_5**
C_1	0.62						
C_2	0.07	0.08					
C_3	0.01	0.01	0.69				
C_{4a}	0.02	0.002	0.45	0.61			
C_{4b}	0.05	0.06	0.55	0.59	0.19		
C_5	0.01	0.003	0.39	0.43	0.89	0.25	
C_6	0.31	0.15	0.81	0.28	0.21	0.89	0.13

Figure 3.8.24 APG-derived Venous Volume (ml) in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



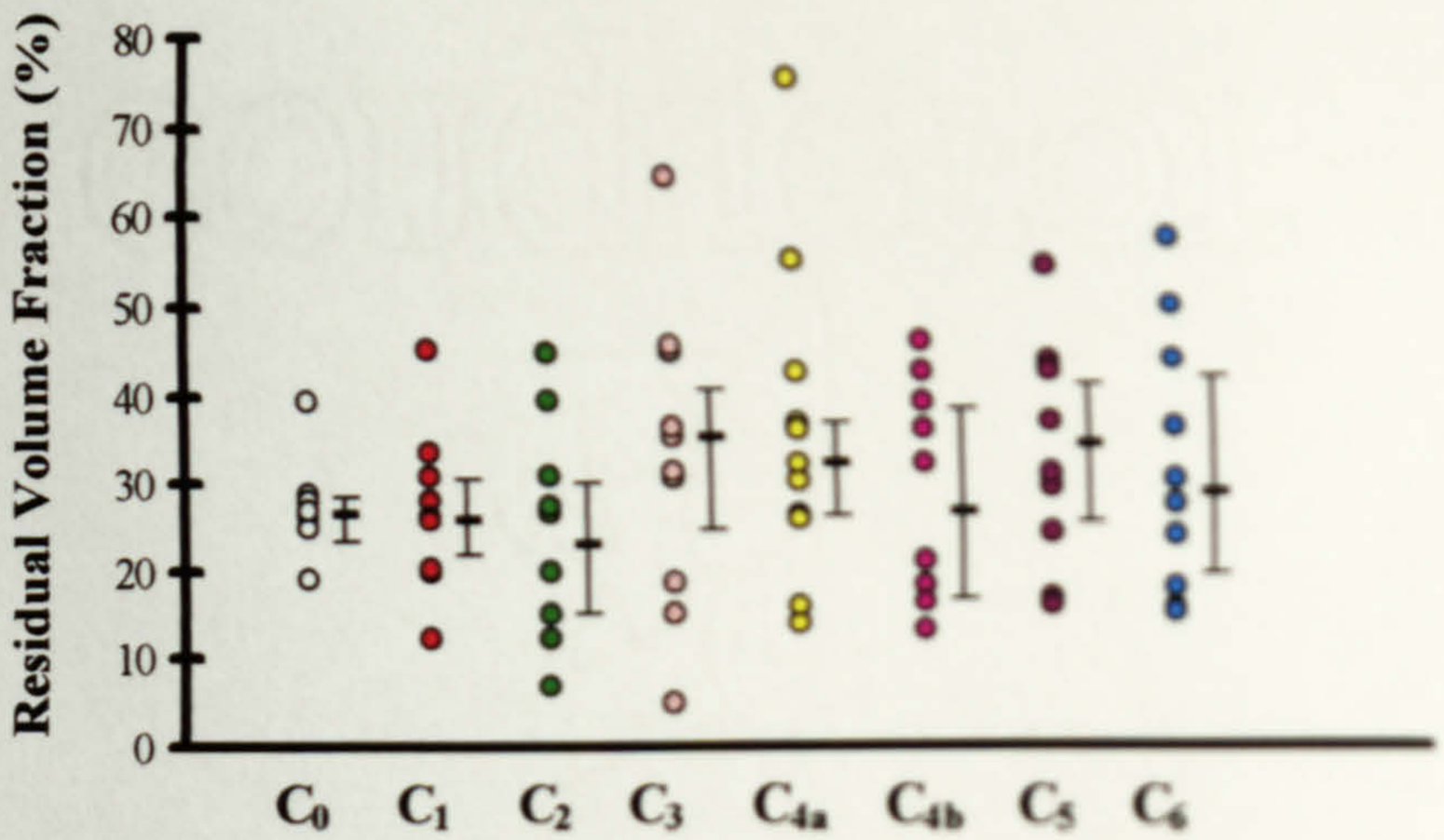
APG-Derived Ejection Fraction 2-Tailed P values Corrected for ties (Mann-Whitney U Test)							
CEAP classification	**C_0**	**C_1**	**C_2**	**C_3**	**C_{4a}**	**C_{4b}**	**C_5**
C_1	0.47						
C_2	0.37	0.93					
C_3	0.03	0.04	0.13				
C_{4a}	0.003	0.001	0.001	0.07			
C_{4b}	0.15	0.22	0.32	0.83	0.19		
C_5	0.003	0.001	0.002	0.12	0.90	0.21	
C_6	0.01	0.01	0.01	0.39	0.45	0.36	0.49

Figure 3.8.25 APG-derived Ejection Fraction (%) in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



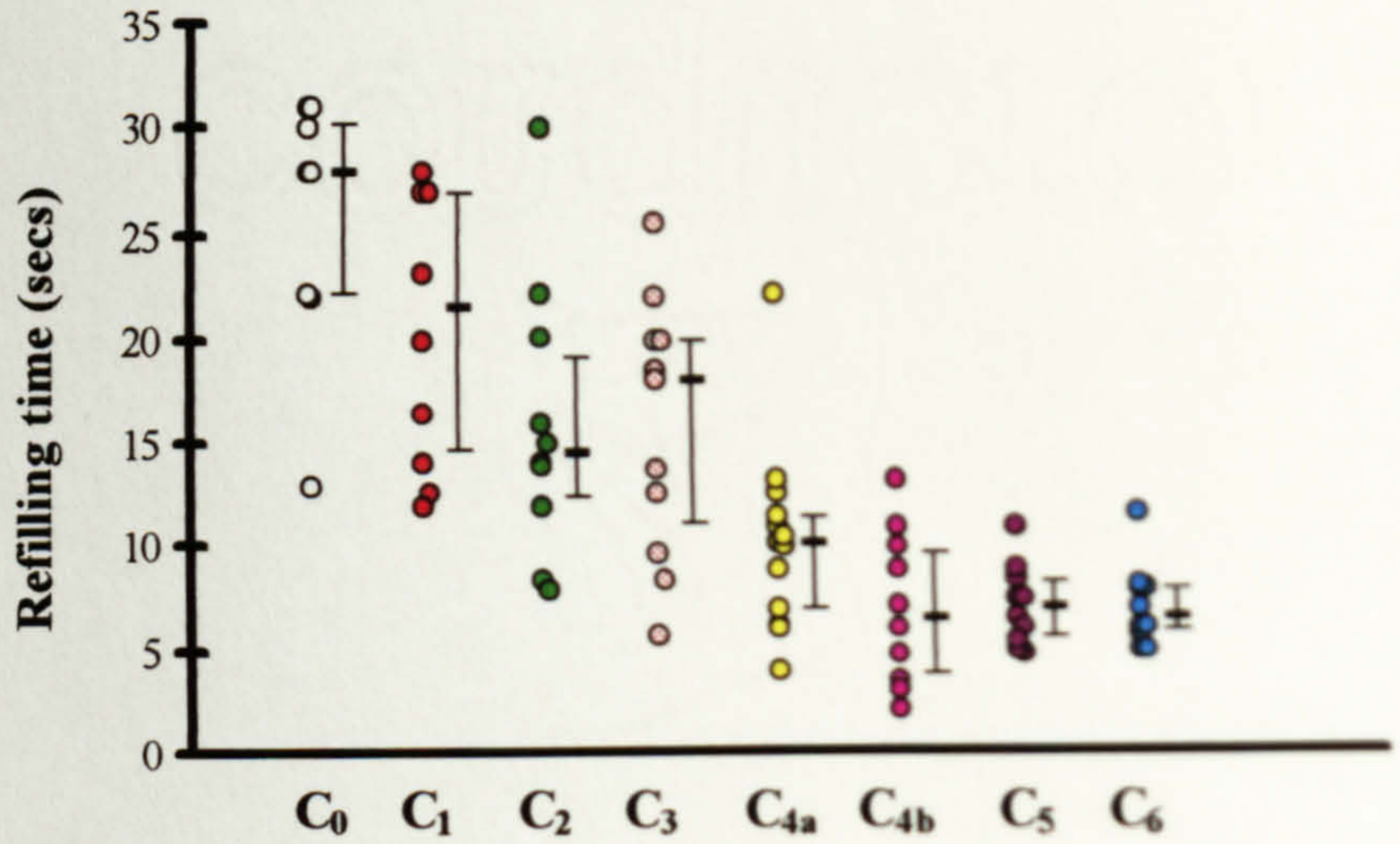
APG-Derived Ejected Volume 2-Tailed P values Corrected for ties (Mann-Whitney U Test)							
CEAP classification	**C_0**	**C_1**	**C_2**	**C_3**	**C_{4a}**	**C_{4b}**	**C_5**
C_1	1.00						
C_2	0.21	0.17					
C_3	0.50	0.18	0.72				
C_{4a}	0.94	0.57	0.21	0.19			
C_{4b}	0.92	0.74	0.19	0.23	0.90		
C_5	0.85	0.51	0.25	0.32	0.70	0.87	
C_6	0.78	1.00	0.15	0.27	0.95	0.93	0.82

Figure 3.8.26 APG-derived Ejected Volume (ml) in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



APG-Derived Residual Volume Fraction 2-Tailed P values Corrected for ties (Mann-Whitney U Test)							
CEAP classification	**C_0**	**C_1**	**C_2**	**C_3**	**C_{4a}**	**C_{4b}**	**C_5**
C_1	1.00						
C_2	0.59	0.49					
C_3	0.21	0.20	0.13				
C_{4a}	0.12	0.10	0.09	0.88			
C_{4b}	0.92	0.87	0.36	0.62	0.42		
C_5	0.21	0.25	0.09	0.94	0.97	0.44	
C_6	0.65	0.59	0.19	0.72	0.55	0.70	0.59

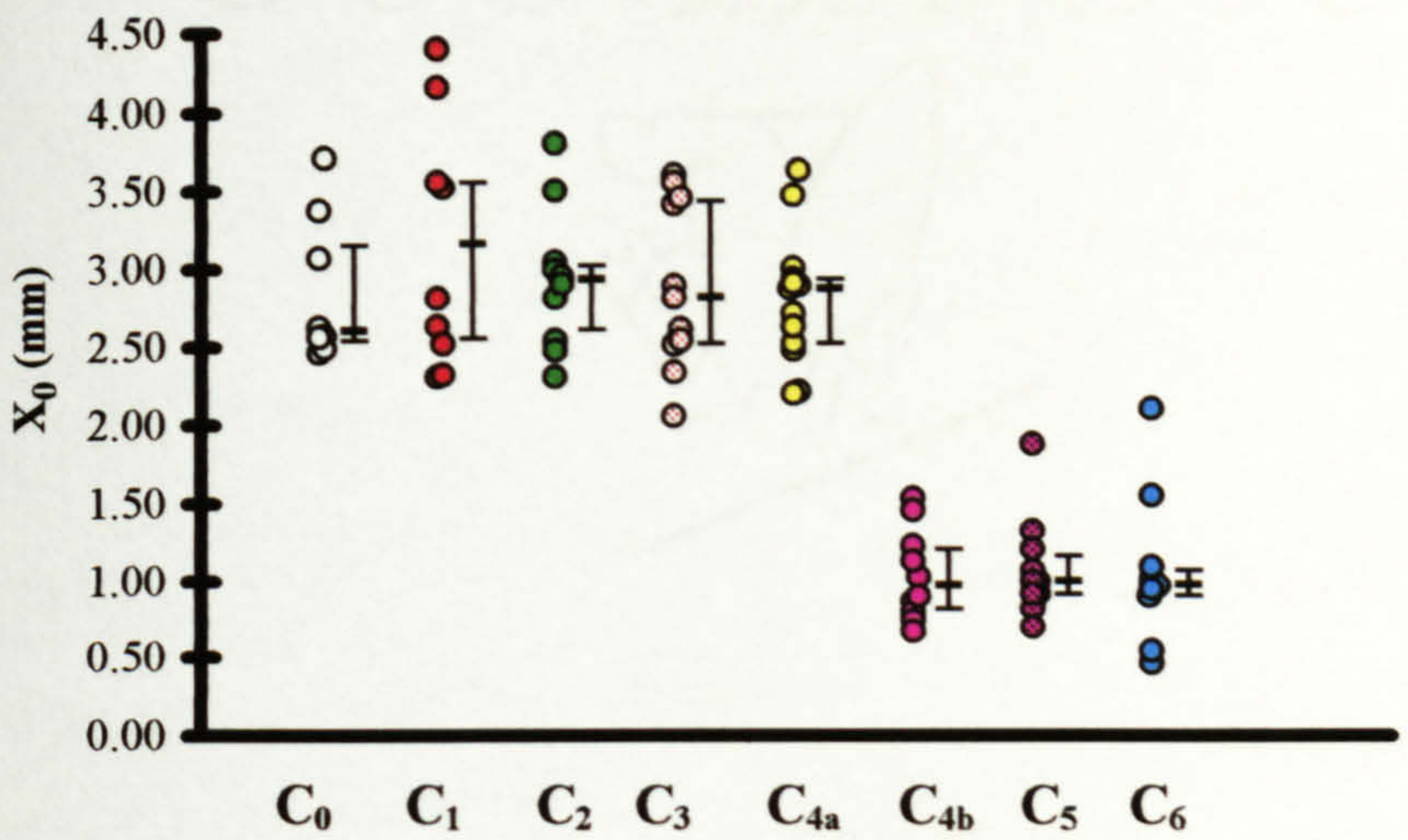
Figure 3.8.27 APG-derived Residual Volume Fraction (%) in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.



PPG Refilling Times (Secs) with No Cuffs Applied 2-Tailed P values Corrected for ties (Mann-Whitney U Test)							
CEAP classification	**C_0**	**C_1**	**C_2**	**C_3**	**C_{4a}**	**C_{4b}**	**C_5**
C_1	0.07						
C_2	0.01	0.15					
C_3	0.004	0.11	0.94				
C_{4a}	0.0004	0.0005	0.01	0.04			
C_{4b}	0.0005	0.0003	0.001	0.003	0.07		
C_5	0.0004	0.0002	<0.0005	0.001	0.04	0.76	
C_6	0.0004	0.0002	0.0005	0.001	0.03	0.82	0.93

Figure 3.8.28 Refilling times with no cuffs in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.

Tissue tonometry Results



Distance travelled (X_0 (mm)) by Plunger in Initial Fast Indentation Phase I 2-Tailed P values Corrected for ties (Mann-Whitney U Test)							
CEAP classification	**C_0**	**C_1**	**C_2**	**C_3**	**C_{4a}**	**C_{4b}**	**C_5**
C_1	0.35						
C_2	0.48	0.69					
C_3	0.66	0.72	0.74				
C_{4a}	0.79	0.45	0.53	0.88			
C_{4b}	0.0004	0.0002	0.0002	0.0001	0.0001		
C_5	0.0004	0.0002	0.0002	0.0001	0.0001	0.56	
C_6	0.0004	0.0002	0.0002	0.0001	0.0001	0.68	0.85

Figure 3.8.29 Distance travelled by plunger (mm) during phase I in normal subjects and in patients with CVD classified according to the CEAP method of classification with the 2-Tailed P values corrected for ties (Mann-Whitney U test) applied to test for statistical significance between the different CEAP groups.

Tissue Tonometry Data for Distance Travelled (X_0) in mm during Initial Fast Indentation Phase I (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	X_0	C_1	X_0	C_2	X_0	C_3	X_0	C_{4a}	X_0	C_{4b}	X_0	C_5	X_0	C_6	X_0
Median	2.6	Median	3.2	Median	2.9	Median	2.8	Median	2.9	Median	1.0	Median	1.0	Median	1.0
LQ	2.5	LQ	2.6	LQ	2.6	LQ	2.5	LQ	2.5	LQ	0.8	LQ	0.9	LQ	0.9
UQ	3.2	UQ	3.6	UQ	3.0	UQ	3.4	UQ	2.9	UQ	1.2	UQ	1.2	UQ	1.1
Tissue Tonometry Data for Distance Travelled (X_I-X_0) in mm during Slower Indentation Phase II (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	X_I-X_0	C_1	X_I-X_0	C_2	X_I-X_0	C_3	X_I-X_0	C_{4a}	X_I-X_0	C_{4b}	X_I-X_0	C_5	X_I-X_0	C_6	X_I-X_0
Median	0.3	Median	0.3	Median	0.4	Median	0.5	Median	0.7	Median	0.7	Median	0.5	Median	0.5
LQ	0.2	LQ	0.2	LQ	0.4	LQ	0.5	LQ	0.5	LQ	0.6	LQ	0.3	LQ	0.4
UQ	0.3	UQ	0.4	UQ	0.7	UQ	0.6	UQ	0.8	UQ	0.9	UQ	0.8	UQ	0.9
Tissue Tonometry Data for Rate Constant Parameter (-1/Tau) in $secs^{-1}$ during Slow Indentation Phase II (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	-1/Tau	C_1	-1/Tau	C_2	-1/Tau	C_3	-1/Tau	C_{4a}	-1/Tau	C_{4b}	-1/Tau	C_5	-1/Tau	C_6	-1/Tau
Median	2.5	Median	2.5	Median	2.7	Median	3.3	Median	3.1	Median	3.5	Median	2.5	Median	3.2
LQ	2.3	LQ	2.1	LQ	2.1	LQ	2.8	LQ	2.7	LQ	2.2	LQ	2.2	LQ	2.5
UQ	2.7	UQ	3.0	UQ	3.4	UQ	4.2	UQ	4.4	UQ	4.8	UQ	3.5	UQ	3.6
Tissue Tonometry Data for Distance Travelled (X_I-X_0) in mm during Slower Indentation Phase III (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	X_I-X_0	C_1	X_I-X_0	C_2	X_I-X_0	C_3	X_I-X_0	C_{4a}	X_I-X_0	C_{4b}	X_I-X_0	C_5	X_I-X_0	C_6	X_I-X_0
Median	0.2	Median	0.2	Median	0.3	Median	0.5	Median	0.7	Median	0.5	Median	0.2	Median	0.7
LQ	0.1	LQ	0.1	LQ	0.2	LQ	0.3	LQ	0.6	LQ	0.1	LQ	0.1	LQ	0.3
UQ	0.3	UQ	0.2	UQ	0.4	UQ	0.5	UQ	0.8	UQ	0.6	UQ	0.7	UQ	1.0
Tissue Tonometry Data for Rate Constant Parameter (-1/Tau) in $secs^{-1}$ during Slow Indentation Phase III (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	-1/Tau	C_1	-1/Tau	C_2	-1/Tau	C_3	-1/Tau	C_{4a}	-1/Tau	C_{4b}	-1/Tau	C_5	-1/Tau	C_6	-1/Tau
Median	75.3	Median	127.4	Median	80.1	Median	89.6	Median	50.2	Median	56.6	Median	81.2	Median	71.0
LQ	56.7	LQ	72.3	LQ	68.4	LQ	76.4	LQ	61.0	LQ	51.0	LQ	54.4	LQ	56.0
UQ	157.6	UQ	424.8	UQ	126.2	UQ	104.6	UQ	55.7	UQ	125.5	UQ	437	UQ	114.4

Table 3.8.1 Median and inter-quartile ranges for tissue tonometry-derived distance/rate constant parameters for the sample population in this study.

Tissue Tonometry Data for Series Hooke Element (Spring Constant) C_2 in g/mm during Phase I (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	C_2	C_1	C_2	C_2	C_2	C_3	C_2	C_{4a}	C_2	C_{4b}	C_2	C_5	C_2	C_6	C_2
Median	1.3	Median	1.1	Median	1.1	Median	1.2	Median	1.2	Median	3.5	Median	3.3	Median	3.4
LQ	1.1	LQ	0.9	LQ	1.1	LQ	1.0	LQ	1.1	LQ	2.8	LQ	2.8	LQ	3.1
UQ	1.3	UQ	1.3	UQ	1.3	UQ	1.3	UQ	1.3	UQ	4.1	UQ	3.6	UQ	3.7
Tissue Tonometry Data for Series Kelvin Element (Spring Constant) C_1 in g/mm during Phase II (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	C_1	C_1	C_1	C_2	C_1	C_3	C_1	C_{4a}	C_1	C_{4b}	C_1	C_5	C_1	C_6	C_1
Median	12.0	Median	10.3	Median	7.7	Median	6.3	Median	4.9	Median	4.5	Median	7.1	Median	6.2
LQ	10.6	LQ	8.0	LQ	5.2	LQ	5.2	LQ	4.0	LQ	3.5	LQ	4.4	LQ	3.7
UQ	15.2	UQ	17.6	UQ	9.3	UQ	7.3	UQ	6.2	UQ	5.1	UQ	10.0	UQ	8.8
Tissue Tonometry Data for Dashpot Constant, k , in g/(mm/sec) during Phase II (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	k	C_1	k	C_2	k	C_3	k	C_{4a}	k	C_{4b}	k	C_5	k	C_6	k
Median	29.2	Median	25.4	Median	20.9	Median	20.5	Median	19.8	Median	16.8	Median	20.1	Median	19.9
LQ	26.3	LQ	17.5	LQ	12.4	LQ	18.9	LQ	13.3	LQ	10.9	LQ	12.0	LQ	12.4
UQ	34.5	UQ	46.7	UQ	32.5	UQ	24.6	UQ	35.0	UQ	17.9	UQ	25.1	UQ	29.2
Tissue Tonometry Data for Series Kelvin Element (Spring Constant) C_1 in g/mm during Phase III (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	C_1	C_1	C_1	C_2	C_1	C_3	C_1	C_{4a}	C_1	C_{4b}	C_1	C_5	C_1	C_6	C_1
Median	17.6	Median	22.2	Median	9.9	Median	7.3	Median	4.8	Median	6.2	Median	20.9	Median	5.2
LQ	11.9	LQ	15.7	LQ	7.8	LQ	6.6	LQ	4.0	LQ	5.2	LQ	5.5	LQ	3.3
UQ	34.8	UQ	43.7	UQ	13.5	UQ	10.2	UQ	5.6	UQ	23.5	UQ	41.8	UQ	9.7
Tissue Tonometry Data for Dashpot Constant, k , in g/(mm/sec) during Phase III (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
C_0	k	C_1	k	C_2	k	C_3	k	C_{4a}	k	C_{4b}	k	C_5	k	C_6	k
Median	2279	Median	3566	Median	889	Median	728	Median	294	Median	709	Median	1945	Median	400
LQ	947	LQ	1331	LQ	571	LQ	578	LQ	276	LQ	263	LQ	640	LQ	271
UQ	3830	UQ	34968	UQ	1790	UQ	1076	UQ	384	UQ	1548	UQ	10624	UQ	446

Table 3.8.2 Median and inter-quartile ranges for the spring and dashpot constants for the sample population in this study.

Correlation Results

r value	APG-derived parameters versus Duplex-derived parameters
0.25	APG-derived Venous Filling Index and Duplex-derived total Reflux Volume.
0.04	APG-derived Venous Filling Index and duplex-derived total valve closing time.
-0.33	APG-derived Venous Filling Index and Duplex-derived total Doppler Efficiency Index.
0.15	APG-derived Venous Filling Index and Duplex-derived total Reflux Time Ratio.
0.12	APG-derived Venous Filling Index and Duplex-derived total Peak Reflux Velocity Ratio.
-0.41	APG-derived Venous Filling Index and PPG-derived Venous Refilling Times.
-0.05	APG-derived Arterial Inflow and Doppler-derived Resting Ankle Brachial Pressure Indices.
-0.19	PPG-derived Venous Refilling Times and Duplex-derived total Reflux Time Ratio.
-0.01	PPG-derived Venous Refilling Times and Duplex-derived total Reflux Volume.
-0.28	PPG-derived Venous Refilling Times and Duplex-derived total valve closing time.

Table 3.8.3 Regression analysis between PPG-derived, APG-derived and duplex-derived parameters to calculate the correlation value between the methodologies.

r value	**Tonometry-derived parameter of skin compliance versus APG-derived parameters, Duplex-derived parameters, PPG-derived parameters, CEAP scoring parameters and patient data.**
-0.1	Distance travelled, X_0, during phase I and APG-derived outflow fraction.
-0.14	Distance travelled, X_0, during phase I and APG-derived venous volume.
-0.34	Distance travelled, X_0, during phase I and APG-derived venous filling index.
0.04	Distance travelled, X_0, during phase I and APG-derived ejection volume.
0.21	Distance travelled, X_0, during phase I and APG-derived ejection fraction.
-0.06	Distance travelled, X_0, during phase I and APG-derived residual volume fraction.
-0.37	Distance travelled, X_0, during phase I and APG-derived arterial inflow.
0.57	Distance travelled, X_0, during phase I and PPG-derived venous refilling time (no cuffs).
-0.07	Distance travelled, X_0, during phase I and duplex-derived total valve closing time.
-0.61	Distance travelled, X_0, during phase I and diameter of incompetent veins.
0.08	Distance travelled, X_0, during phase I and patient height.
-0.36	Distance travelled, X_0, during phase I and patient age.
-0.27	Distance travelled, X_0, during phase I and patient weight.
-0.18	Distance travelled, X_0, during phase I and patient body surface area.
0.32	Distance travelled, X_0, during phase I and patient body mass index.
0.12	Distance travelled, X_0, during phase I and duplex-derived reflux fraction.
-0.01	Distance travelled, X_0, during phase I and duplex-derived total reflux volume.
0.27	Distance travelled, X_0, during phase I and duplex-derived peak reflux velocity ratio.
0.13	Distance travelled, X_0, during phase I and duplex-derived reflux time ratio.
-0.12	Distance travelled, X_0, during phase I and duplex-derived Doppler efficiency index.
-0.75	Distance travelled, X_0, during phase I and CEAP clinical score.
-0.59	Distance travelled, X_0, during phase I and CEAP disability score.
-0.48	Distance travelled, X_0, during phase I and CEAP anatomical score.

Table 3.8.4 Regression analysis between tonometry-derived parameter of the skin compliance, duplex-derived parameters, PPG-derived parameters, CEAP scoring parameters and patient data to calculate the correlation value between the different methodologies.

3.8.5. Discussion

In this study the purpose of the investigation was to establish the relationship between the severity of the venous disease indicated by the clinical classification (CEAP) and duplex ultrasound and plethysmographic methods of investigating the function of the venous system. A number of authors have investigated this problem previously but no study has tested the CEAP system in this way and neither has the clinical status been checked using the tonometry technique to assess skin compliance which I have shown in earlier chapters is a very reliable indicator of the severity of venous disease, when used in the correct way.

The use of duplex ultrasound scanning in investigating valvular dysfunction in the superficial, deep and perforating veins has suggested that they all contribute to a critical level of total reflux and that reflux in the popliteal and tibial veins correlated well with the severity of disease (Vasdekis et al, 1989). Using a standardised cuff deflation technique, they demonstrated that there was a high incidence of skin changes in limbs with a cumulative peak reflux greater than 10ml/sec. They calculated the peak reflux volume flow at the time of peak reflux velocity. The diameter of the vein at the site of reflux is measured using B-mode instrumentation and the peak reflux velocity (PRV) is determined from the velocity waveform after cuff deflation obtained from the spectral Doppler analysis using pulse-wave Doppler mode instrumentation.

In the sample population investigated, 44% had SVI only and 22% had a combination of SVI and DVI. These figures were similar to those of Labropoulos et al 1995 and Shami et al 1993. Interestingly in my study population none of the patients with skin changes was as a result of DVI alone compared to 11% (Labropoulos et al, 1995); and 15% (Shami et al, 1993) in earlier studies. The number of patients with skin changes due to perforator incompetence only was 2.4% in my sample population, which was very similar to Labropoulos et al sample population with 3% (Labropoulos et al, 1995).

The duplex-derived reflux fraction parameter in the superficial veins were significantly higher in the groups (C_2-C_6) with evidence of significant reflux (i.e. reflux lasting longer than 0.5 seconds) as compared to the C_0 and C_1 groups which have no evidence of significant reflux (i.e. reflux lasting less than 0.5 seconds). There was no statistically significant difference in this parameter between the C_2 and C_6 groups and between the C_0 and C_1 groups (Figure 3.8.1).

There was a significant increase in the reflux fraction parameter of the incompetent deep venous system segments in patients in the C_3 to C_6 groups as compared to those in the

C_0, C_1 and C_2 groups with no evidence of deep venous incompetence. There was no significant difference in this parameter between the C_0, C_1 and C_2 groups as there were no patients with incompetent deep veins in the C_2 patient group. Similarly, there were no significant differences between the groups with deep venous incompetence except for the C_{4a} and C_{4b} groups (p=0.05) and the C_{4b} and C_6 groups (p=0.04) which showed some significance though there was considerable overlap (Figure 3.8.2).

The duplex-derived reflux volume flow parameter in the superficial veins were significantly higher in the groups (C_2-C_6) with evidence of significant reflux (i.e. reflux lasting longer than 0.5 seconds) as compared to the C_0 and C_1 groups which have no evidence of significant reflux (i.e. reflux lasting less than 0.5 seconds). There was no statistically significant difference in this parameter between the C_0 and C_1 groups and between the C_3 and C_6 groups but in the C_2 group, the reflux volume flow was moderately reduced as compared to the other groups with reflux with the exception of the C_5 group though there was a wide scatter in the data points between these groups. (Figure 3.8.3). This would suggest that in the C_2 group the amount of venous reflux was not as extensive as in the other groups with more extensive clinical sequelae.

There was a significant increase in the reflux volume flow parameter of the incompetent deep venous system segments in patients in the C_3 to C_6 groups as compared to those in the C_0, C_1 and C_2 groups with no evidence of deep venous incompetence. There were no significant differences in this parameter between the C_0, C_1 and C_2 groups as there were no patients with incompetent deep veins in the C_2 patient group. Similarly, there were no significant differences between the groups with deep venous incompetence except for the C_{4a} and C_5 groups (p=0.05) which showed some significance though there was considerable overlap (Figure 3.8.4).

The duplex-derived peak reflux velocity ratio in the superficial veins were significantly higher in the C_2 to C_6 groups with significant reflux as compared to the C_0 and C_1 groups with no significant reflux. Again, there were no significant differences in this parameter between the C_2 and C_6 groups and the C_0 and C_1 groups (Figure 3.8.5).

There was a significant increase in the duplex-derived reflux velocity ratio parameter of the incompetent deep venous system segments in patients in the C_3 to C_6 groups as compared to those in the C_0, C_1 and C_2 groups with no evidence of deep venous incompetence. There were no significant differences in this parameter between the C_0, C_1 and C_2 groups as there were no patients with incompetent deep veins in the C_2 patient group. Similarly, there were no significant differences between the groups with

deep venous incompetence except for the C_{4a} group (p=0.03) which had a lower value amongst the C_3 to C_6 groups but there was considerable overlap in the data (Figure 3.8.6).

The duplex-derived reflux time ratio parameter in the superficial veins were significantly higher in the groups (C_2-C_6) with evidence of significant reflux (i.e. reflux lasting longer than 0.5 seconds) as compared to the C_0 and C_1 groups which have no evidence of significant reflux (i.e. reflux lasting less than 0.5 seconds). The reflux time ratio was calculated by dividing the valve closing time by the valve opening time obtained from the spectral Doppler traces as described earlier. There were no statistically significant differences in this parameter between the C_2 and C_6 groups though there was moderate differences between the C_2 and C_3 groups compared with the more severe groups though with some overlap present in the data points. There were no significant differences between the C_0 and C_1 groups (Figure 3.8.7).

There was a significant increase in the duplex-derived reflux time ratio parameter of the incompetent deep venous system segments in patients in the C_3 to C_6 groups as compared to those in the C_0, C_1 and C_2 groups with no evidence of deep venous incompetence. There were no significant differences in this parameter between the C_0, C_1 and C_2 groups as there were no patients with incompetent deep veins in the C_2 patient group. Similarly, there were no significant differences between the groups with deep venous incompetence except for the C_5 and C_6 groups which had a lower value amongst the C_3 to C_6 groups but with considerable overlap in the data (Figure 3.8.8).

Other studies (Beckwith et al, 1993) which have recently been described proposed the measurement of the Doppler curves by analysing the volume of the wave of blood using the Psathakis's index (Psathakis et al, 1987) which is a Doppler Efficiency Index (EI d). In my study the Doppler efficiency index, EI d, in the superficial venous system were significantly higher in the C_0 and C_1 groups as compared to the C_2 to C_6 groups indicating that the valves are more efficient in preventing reflux flow, i.e. having competent valves, in the C_0 and C_1 groups compared to the groups with significant reflux. There were no significant differences between the C_0 and C_1 groups but there was wide scatter of data between the C_2 and C_6 groups (Figure 3.8.9).

The Doppler efficiency index, EI d, in the deep venous system were significantly higher in the C_0 , C_1 and C_2 groups as compared to the C_3 to C_6 groups again indicating that the valves are more efficient in preventing reflux flow, i.e. having competent valves, in the C_0, C_1 and C_2 groups compared to the groups with significant reflux. There were no

significant differences between the C_0 , C_1 and C_2 groups but there was wide scatter of data between the C_3 and C_6 groups (Figure 3.8.10).

There was a significant increase in the duplex-derived total reflux volume flow parameter (figure 3.8.11) and in the duplex-derived total valve closing time parameter (figure 3.8.12) in all incompetent segments in patients in the C_2 to C_6 groups as compared to those in the C_0 and C_1 groups with no significant differences present in these parameters between the C_2 to C_6 patient groups.

In summary, duplex ultrasonography applied to the superficial venous system appears to group the veins into those with reflux and those without. None of the quantitative assessments of incompetence revealed increased venous reflux in the more severely affected groups. Similarly in the deep veins, patients with the lesser stages of venous disease had no significant venous reflux, and all those with the more severe stages C_3 – C_6 had similar levels of venous reflux, with no evidence of more severe reflux in the more severe clinical stages of venous disease. These findings indicate that duplex ultrasonography is useful in detecting venous reflux, but ultrasound assessments of venous blood flow seem to add nothing to the assessment.

There were no significant differences in the diameters of the superficial veins as measured by B-mode ultrasound, between the C_0 and C_1 groups. However, there was a statistically significant increase in the diameters of the superficial veins between these two groups with no significant reflux and the C_2 to C_6 groups with evidence of significant reflux. There was great overlap between the data points in the C_2 to C_6 groups (Figure 3.8.13). These findings again show that patients with the more severe stages of venous disease have larger veins than those with trivial or no venous disease.

No trend in patient height was seen between the C_0 and C_6 groups with a very wide scatter of data points in this sample population (Figure 3.8.14). Patient height is unimportant in determining the severity of the venous syndrome.

There was a wide scatter in the body weight (Kg) parameter between the C_0 and C_6 groups but patients in groups C_2 to C_6 were significantly heavier than in groups C_0 and C_1. Patient weight is sometimes blamed as the cause for varicose veins and there is some evidence to support this suggestion from this study. However, patients in the more severe clinical stages were also older than those in the C_0 and C_1 groups, which might also account for the differences (Figure 3.8.15).

The clinical score increased almost linearly with the progression of the disease as more symptoms were included in the scoring thus reflecting the severity of the disease. There

were no significant differences between the C_0 and C_1 groups or the C_2 and C_3 groups (Figure 3.8.16). This aspect of the CEAP system appears to reflect the severity of the venous disease more satisfactorily than many of the objective indices!

There was a significant increase in the anatomical score between the normal control groups (C_0 and C_1) and the disease groups (C_2 to C_6) as more segments of the venous system were identified as incompetent. There was a significant difference between the C_2 and C_3 groups but not between the C_3 and C_6 groups indicating that the number of segments involved does not correlate with the severity of the disease (Figure 3.8.17).

Similarly for the disability scoring, there was a significant increase in the disability score between the normal control groups (C_0 and C_1) and the disease groups (C_2 to C_6). However, there was a significant difference between the C_2 , C_3 , C_{4a} groups and the C_{4b}, C_5, C_6 groups indicating the extent of the disability as related to the severity of the disease (Figure 3.8.18).

There was a wide overlap in the data for the body mass index between the groups from C_0 to C_6 though the trend show an increase in this parameter with the severity of the disease with the exception of the C_{4a} group, possibly reflecting that obesity is a risk factor (Figure 3.8.19). Again, the greater age of patients in the more severely affected groups makes direct comparison unreliable. Interestingly, only a few of the patients could be classified as obese (BMI >30).

As for the body surface area parameter, there was no significant difference between any of the groups with a wide scatter of the data points present between the groups. However, there is a trend indicating an increase in the body surface area in the patient groups as compared to the normal controls (Figure 3.8.20).

The haemodynamic characteristics associated with progressive chronic venous disease have also been investigated with the parameters obtained from APG (Welkie et al, 1992). They demonstrated that deterioration in venous haemodynamics paralleled the clinical severity of chronic venous disease through to the International Society for Cardiovascular Surgery (ISCVS) class 2 group (brawny induration and hyperpigmentation) and that once brawny oedema and hyper-pigmentation had occurred, ulceration can occur without additional deterioration of venous macrohaemodynamics thereby suggesting that progressive clinical deterioration may be the result of microcirculatory changes.

In my study population, there was great overlap in the outflow fraction between the groups with no significant difference in this parameter between the normal controls and

the patient groups (Figure 3.8.21). The outflow fraction are greater than 35% with superficial compression for all the groups. This was not surprising because in the patient population in this study, there was no evidence of deep venous obstruction on duplex scanning.

Outflow tests are performed to detect chronic obstructive disease. A reduced outflow fraction indicates the presence and severity of the obstruction. Studies using APG to quantify chronic venous outflow obstruction and venous outflow resistance (Nicolaides et al, 1993) has shown that in limbs with mild to moderate venous obstruction, the outflow fraction was 30-38% and in limbs with severe obstruction the outflow fraction was less than 30% of the venous capacitance. In the case of chronic obstruction testing the degree of collateralisation of the superficial veins can also be measured. Since collateralised veins may hide the presence of obstructed deep veins, the test is first performed with the compression of the superficial veins and if this proves to be normal then a second outflow test to determine degree of collateralisation without compression of the superficial veins is not necessary. The degree of superficial collateralisation is measured when the outflow test is performed with compression of the long saphenous vein (Rulo et al, 1989; Christopoulos et al, 1989). Therefore, normal values for outflow fraction are greater than 35% with superficial compression and greater than 40% without superficial compression. Limbs without obstruction will empty at least 40% of the retained blood volume one second after deflation of a proximally placed tourniquet.

There was great overlap in the APG-derived arterial inflow parameter in millilitres per minute between the normal controls and the patient groups. However, there was a statistically significant increase in the arterial inflow between the normal controls C_0 group and the patient groups, C_1 to C_6. There was also a moderate increase in this parameter between the C_1 group and the more severely diseased groups C_{4b} and C_5 but not between the C_2, C_3, C_{4a} groups and the C_6 group (Figure 3.8.22). Again, the sample numbers were small but the trend showed an increase in the arterial inflow with increasing severity of venous disease. This observation has been reported previously in a number of papers in which laser Doppler fluxmetry has been used to assess the cutaneous circulation. The increased blood flow is perhaps a response to the chronic inflammatory processes which involve the limb in patients with chronic venous disease. Other studies have shown this parameter to be higher in patients with severe chronic venous disease. In a study to assess the venous hypertensive microangiopathy in relation to clinical severity (Christopoulos et al, 1991), arterial inflow and perimalleolar skin

blood flow were measured with venous-occlusion air plethysmography and laser Doppler flowmetry . They showed that there was a marked increase in the resting arterial inflow in limbs with complicated varicose veins, skin changes and deep venous incompetence as compared to normal limbs and limbs with uncomplicated varicose veins. More recently, another study (Christopoulos et al, 1995) in which the haemodynamic effect of venous hypertension in the lower limb microcirculation was studied, an increase in the resting arterial inflow with increasing incidence of ulceration was found. This was considered to reflect the damage in the microcirculation produced by chronic venous hypertension.

There was a significant increase in the APG-derived venous filling index parameter, which is a measurement of the amount of reflux present in the whole limb by measuring the average filling rate of the veins, between the groups with no significant evidence of reflux present (C_0 and C_1) and the groups with significant evidence of reflux (C_2 to C_6). The median VFI increased between the C_2 group and the groups with increasing severity of the disease (C_3, C_{4a}, C_{4b}, C_5 and C_6), although statistical significance was not reached because of the wide scatter between the groups. There was no difference between the C_3 and C_6 groups (Figure 3.8.23). In other words, the VFI parameter is very good at differentiating between normal controls and patients with significant reflux but cannot differentiate between the disease groups suggesting that the amount of reflux flow present in the disease groups has no further effect at the microcirculatory level once the clinical sequelae of the disease have been established. This clearly demonstrates that VFI assesses the severity of venous reflux, but that this does not increase in the more severely affected patients. Some other factor is responsible for the development of severe skin changes and leg ulceration. Alternatively, it is possible that the severity of the skin changes limits exercise in these patients preventing reliable assessment of the VFI.

Other studies correlating APG-derived VFI parameter with the clinical severity of chronic venous disease (Christopoulos et al, 1988) have shown that there was an increased incidence of sequelae with increasing values of the venous filling index (VFI). Normal limbs will show a VFI of between 0.6 to 1.7 ml/sec and represents the venous filling rate from the capillaries at rest as a result of the arterial inflow. A VFI of greater than 2.0 ml/sec indicates the presence of venous reflux in addition to the arterial inflow. There was great overlap between the groups with no significant difference in the venous volume parameter between the normal controls and the patient groups (Figure 3.8.24)

but there was an increasing trend from the normal controls to the disease groups. This suggests an expansion of the venous compartment in patients with venous disease, and is again consistent with previously published data.

There was great overlap in the ejection fraction parameter, which provides a measure of the effectiveness of the calf muscle pump, between the normal control group and the disease groups with moderately significant decrease in this parameter between the C_0, C_1 and C_2 groups as compared to the C_{4a} and C_5 groups. However, the general trend was a decrease between the normal controls and the patient groups (Figure 3.8.25). One would expect this parameter to be significantly reduced in patients with significant disease as compared to normal controls. This was not the case in this study population possibly due to bias in the study design in which 'fit' patients are selected who can handle the rigours of the air plethysmography procedures. Also since there were no significant outflow obstruction identified in any group with duplex scanning and the ejection fraction showed similar values within the normal range, this suggested that there were no impaired calf muscle pumps among the sample population studied.

Similarly, there were no significant differences in the APG-derived ejected volume between the normal controls and patient groups ((Figure 3.8.26).

Limbs of patients with superficial venous insufficiency will show a below normal EF mainly due to an increased venous volume. A poor calf muscle pump will have an EF less than 40%. In limbs with deep venous disease, a decreased EF is the result of both increased venous volume and decreased ejected volume (Christopoulos et al, 1987).

It has been demonstrated that when EF and VFI data are combined, there is a good correlation to the incidence of ulceration. There is a high incidence of ulceration in limbs with poor calf pump function and severe reflux. Limbs with a good calf pump but severe reflux are somewhat protected from ulceration. Limbs with poor calf pumps are also protected from ulceration if they have more competent valves (Christopoulos et al, 1989).

There was great overlap in the APG-derived residual volume fraction parameter, which assesses lower limb venous hypertension by measuring venous emptying during exercise and is influenced by venous reflux, between the normal control group and the diseased groups with no significant differences in this parameter between the normal control groups and the patient groups. However, the general trend was a moderate increase between the normal controls and the patient groups (Figure 3.8.27).

It has been previously demonstrated that there is a good correlation (r: 0.83) between RVF and simultaneous measurement of the ambulatory venous pressure (AVP) which is achieved by placing a needle in a vein in the dorsum of the foot and attached to a pressure transducer (Christopoulos et al, 1989) (Welkie et al, 1991).

There was a significant decrease in the PPG-derived refilling times between the normal controls and the disease groups. Also there was a significant decrease in this parameter between the less severe disease groups (C_2 and C_3) and the more severe disease groups (C_{4a}, C_{4b}, C_5 and C_6) (Figure 3.8.28).

This simple and objective test has been validated by venous pressure measurements (Abramowitz et al, 1979; Barnes et al, 1982). Abramowitz et al has shown that the PPG data of refilling times has a very good correlation with venous pressure measurements (93.9%). Correlation of the PPG refilling times and ambulatory venous pressure recovery time has been estimated as 0.88 using a standing exercise and 0.94 with the sitting exercise (Nicolaides et al, 1987). Studies using photoplethysmography have been carried out in the assessment of patients with chronic venous insufficiency (Abramowitz et al, 1979; Barnes et al, 1978). They demonstrated that they could differentiate between superficial and deep vein insufficiency by recording photoplethysmographic changes in the lower calf following brief periods of dorsiflexion of the foot. The reasoning behind the test is that refilling will occur relatively slowly due only to arterial inflow through the muscle in the normal case. If the venous valves are not competent, refilling will occur quickly as blood runs back down the leg veins. In normal subjects, this refilling time is greater than 20 seconds whereas abnormal subjects with incompetent veins will have refilling times of less than 20 seconds. The PPG transducer senses the state of venous filling from the superficial venules in the skin. Perhaps some aspects of its measurement reflects changes in the microcirculation as well as the large vein abnormalities. Photoplethysmography has also been applied clinically to evaluate vein surgery (Pearce et al, 1983; Yao et al, 1986) and compression therapy (Norris et al, 1984; Noyes et al, 1987).

With the advent of new and more sophisticated technology in the form of duplex ultrasound scanning and air plethysmography in assessing patients with chronic venous disease recent studies have highlighted inaccuracies when using photoplethysmography to identify venous reflux. It has been shown that PPG findings do not correlate well with duplex scanning which is now fast becoming the 'Gold standard' in the assessment of chronic venous disease (van Bemmelen et al, 1992). These authors concluded that

though PPG can give an indication of the extent of venous incompetence it cannot distinguish between superficial and deep venous incompetence with the use of a tourniquet and that it provided no additional useful information for the clinician. Another study (Bays et al, 1994) which validated air plethysmography, photoplethysmography and duplex scanning in the evaluation of severe venous stasis found that though PPG was a sensitive method of detecting reflux (100%) its specificity was poor (60%) and that PPG refilling times could not accurately predict the location of reflux.

These inaccuracies may be due to the fact that photoplethysmography can give a measure only of the venous refilling time which is an indirect measurement of venous reflux. The venous refilling time is measured within the capillary bed which may not reflect overall venous function. Also, the capillary blood content is highly temperature dependent which may be a potential source of error. It also provides no estimation of venous obstruction and therefore no quantitative measure of venous obstruction (Struckmann J, 1994).

The results of the tissue tonometry data are similar to those of study III conclusions for figure 3.8.29 and tables 3.8.1 and 3.8.2.

There is a weak correlation between the severity of reflux measurements and APG- and PPG- derived parameters of reflux quantification. The correlation between the PPG-derived refilling times and the APG-derived VFI parameter was r=-0.41 (Table 3.8.3), that is, an increase in the venous filling index resulted in a shortening of the PPG refilling time, both indicating venous reflux. This is possibly due to the fact that reflux is often found to be present in several vein segments. In the duplex-derived measurement of total reflux, the vein segments with significant reflux lasting longer than 0.5 seconds are added up to give a total reflux value for the limb. However, it is not possible to measure reflux in all the incompetent perforators, muscular veins and deep veins if present becomes it would become a laborious and difficult procedure because one would have to take into account the time factor for measurements as well since the patient's tolerance should be an important factor. Because of these reasons, duplex-derived reflux quantification parameters is only used as a research tool for vessels with significant reflux and not as a routine measurement. On the other hand, the APG-derived venous filling index provides a global evaluation of the severity of reflux in the whole limb and is to be regarded as a more reliable indicator of the severity of the physiological abnormality.

There is a weak correlation between the severity of skin hardness as measured by the tissue tonometer parameter, X_0, and APG-, PPG-, duplex-derived parameters of venous function, the CEAP scoring parameters and patient data. The correlation between the X_0 parameter and the CEAP clinical score was r=-0.75 (table 3.8.4), that is, an increase in the clinical score resulted in a decrease in the distance travelled X_0, both of which reflect increasing severity of the disease.

3.8.6. Conclusions

The APG-derived parameters, PPG-derived parameter and the duplex-derived parameters became abnormal starting between the C_2 and C_3 groups and continuing through to the C_6 groups. No physiological parameter shows an increase in value between the more severely affected groups. Although numbers of subjects in this investigation are relatively small, this confirms that the severity of venous reflux as indicated by these tests does not seem to be the cause of the worsening clinical condition of these patients.

The duplex ultrasound quantitative measures of venous reflux show an all-or-none type of response, with reflux present or not in the deep or superficial veins. This therefore must be regarded as an anatomical test, incapable of providing reliable quantitative measures of venous reflux. APG is clearly superior is in this respect.

The tissue tonometry parameters became abnormal only from the C_{4b} group onwards consistent with the clinical impression that it is these patients who are at greatest risk of the more severe sequelae of venous disease. This does not correlate with any of the parameters of venous physiology and therefore it must be concluded that some other factor is responsible for the development of the lesser to the more severe stages of venous disease. The physiological abnormality must clearly be present, but some additional factor is also required for this to result in the appearance of LDS and leg ulceration. Perhaps this reflects differences in the tissue response to venous hypertension, due to some abnormal sensitivity in microcirculation in these patients.

SECTION IV

General Conclusion

4.1. General Conclusions

Chronic venous disease (CVD) of the lower limbs has been a major problem since ancient times and at present it has severe economic consequences in the western world. Its clinical sequelae range from oedema, haemosiderosis and pigmentation, to gross lipodermatosclerosis (LDS) and venous ulceration with the gaiter area as the site most commonly affected in the lower limb. A vast majority of patients show a wide spectrum of skin changes of varying severity which precede venous ulceration. The extent and severity of the disease can be assessed by clinical and physiological methods using techniques such as plethysmography and ultrasound.

At present, there is no standardised objective method of assessing the mechanical properties and degree of skin change in these patients, so that the response to treatment can be objectively monitored. A tissue tonometer was developed in our laboratory for the objective assessment and quantification of the skin changes seen in these patients by measuring the mechanical properties of the skin.

In study **I**, I standardised the calibration, repeatability and reproducibility of the methodology of the tissue tonometry measurements and investigated whether the tonometer can differentiate between the mechanical properties of the skin in normal controls and in patients with lower limb chronic venous disease. I have shown that the tissue tonometer can objectively distinguish between the varying hardness of skin in patients with lower limb chronic venous disease and that the tissue tonometry measurement of the skin compliance during phase I provided an objective means of assessing skin changes in patients with chronic venous disease. I have also shown that the CEAP clinical classification group C_4 was not adequate when measuring the mechanical properties of skin changes and that it was necessary to differentiate between patients with pigmentation alone and no palpable induration (C_{4a}) and patients with liposclerotic skin changes with palpable induration (C_{4b}) to get a more objective assessment of the skin changes that are present in patients with lower limb chronic venous disease. There was also some evidence that the tissue tonometry measurements of distance and rate constant parameters in the subsequent slower phases II and III may possibly reflect the amount and type of tissue oedema present.

I have developed a tissue tonometer that can provide information on the mechanical properties of the skin which made it necessary to distinguish between patients with liposclerotic skin changes and no palpable induration into group C_{4a} from those with liposclerotic skin changes and palpable induration into C_{4b}. These two groups were

originally classed in the same group as C_4. I believe that if this method of classification is going to be universally accepted and survive, it should be allowed to evolve with the developments in new technology which was the original reason for its creation and should be flexible enough to adapt to the new technologies as it has shown to be in this thesis.

Although a number of the tonometry parameters showed relatively poor repeatability, the measurement reflecting tissue compliance was very satisfactory in this respect with a coefficient of variation of only 8% in repeated measurements. This is much better than almost any other measurement in the assessment of venous disease and I immediately realised that this had potential for use as a surrogate endpoint in the assessment of the outcome of treatments for venous disease. However, several further steps would be required before it was fully evaluated for this purpose.

In study **II**, an *in vitro* model, the sponge-in-glycerol model, was applied to obtain displacement versus time curves so as to apply a simple visco-elastic mathematical model, the Kelvin-Hooke model, on the curves obtained to calculate the spring and dashpot constants to explain its mechanical behaviour. I demonstrated that in the sponge in glycerol model, the application of the Kelvin-Hooke model appeared to fit the data quite well and that this model may be applied *in vivo* to analyse the mechanical properties of the skin of patients with lower limb chronic venous disease using a tissue tonometer.

In Study **III**, I applied the Kelvin-Hooke model to the displacement versus time curves obtained *in vivo* in patients with lower limb chronic venous disease classified according to the CEAP method of classification and demonstrated that it fitted the data quite well in assessing the mechanical properties of the skin of patients with lower limb chronic venous disease. Here, a much wider range of patients was included, incorporating those in all of the CEAP stages. The method of tonometry clearly identified those in the more severe stages of venous disease ($C_{4b} - C_6$), as vastly different from those in the lesser stages of the disease, when using the measure of skin compliance. The other parameters of venous disease assessing venous volume and oedema were less valuable in assessing patients with venous disease.

In study **IV**, I compared the clinical assessment of the severity of liposclerotic skin changes made by an experienced vascular surgeon using the CEAP method with the tissue tonometry parameters of skin compliance in patients with bilateral LDS in order to assess whether the tissue tonometer can differentiate between varying severity of skin

hardness and showed that the tissue tonometry measurements of skin compliance provided an objective way of differentiating between the clinical severity of liposclerotic skin changes. It was apparent from the outcome of this study that the tonometry results mirrored the opinion of an experienced clinician in assessing the severity of skin damage of a series of patients all of whom were affected by lipodermatosclerosis.

In study **V**, I measured the skin and subcutaneous layer thickness in the normal and patient groups presenting with lower limb venous disease using a 7.5 MHz transducer and correlating them to displacement versus time curves obtained using the tissue tonometer within the same groups above. This was to assess whether the skin and subcutaneous layer thickness had any influence on the variability of the distance and rate constant parameters obtained from tissue tonometry. I demonstrated that there was no significant correlation between the ultrasound parameters and tissue tonometry parameters and concluded that ultrasound measurements of skin thickness and subcutaneous tissue thickness using a 7.5 MHz linear array probe prove not to be useful in limbs with oedema or inflammatory disease.

In study **VI**, I objectively measured the effects of compression stockings on the mechanical properties of the skin of patients with lower limb chronic venous disease using a tissue tonometer to assess the skin compliance. I also applied a circumferential method to assess the limb volume before and after compression stocking wear. I demonstrated that the response to compression treatment can be readily assessed by tissue tonometry and circumferential limb volumetry. The results showed that the use of compression by these patients for a period of only six weeks could produce a readily measurable change in skin compliance. The magnitude of the change was small (about 30%) but this might be consistent with a clinically useful change. Due to the great repeatability of this measurement it was effective in assess the response to a widely recognised treatment. The fact that this was not just a spurious observation was confirmed by the measurement of limb volume. Patients in this study were clearly wearing their allocated stockings since they showed a decrease in limb volume of about 10%.

In study **VII**, I assessed the inter-variability between examiners and the ease of use of the application of the recently devised CEAP method of classification and scoring of lower limb venous disease. I showed that there was excellent to very good correlation between different examiners of varying experience in the field and that the classification and scoring method was easy to use. The best correlations were found for the

assessment of those data derived from duplex ultrasonography. All observers used the same duplex data on which to base their classification, and non surprisingly came up with identical CEAP grading. The differences lay in the clinical aspects of the study, emphasising that adequate clinical experience and training is required if consistent clinical scoring is to be produced.

In study **VIII**, I further investigated the recently devised CEAP classification of venous disease which related several aspects of the vein damage to the clinical syndrome to see how the physiology of the venous system, as measured by the many different methods of measurement in venous disease including duplex assessment and plethysmographic techniques were related to the clinical stages of the CEAP classification. I also included the measurement of the mechanical properties of the skin using the tissue tonometer developed in our laboratory.

I also correlated the duplex-derived parameters of lower limb venous reflux quantification with the clinical severity of the disease as classified by the CEAP method, the air plethysmography-derived parameters and photoplethysmography-derived parameters of venous function and arterial perfusion with the clinical severity of the disease as classified by the CEAP method and the tissue tonometry-derived parameters of the mechanical properties of lower limb skin changes in the same population with the clinical severity of the disease as classified by the CEAP method. The correlation between the duplex-derived parameters, plethysmographic parameters and tissue tonometry parameters were also investigated. I demonstrated that the APG-derived parameters, PPG-derived parameter and the duplex-derived parameters became abnormal starting between the C_2 and C_3 groups and continuing through to the C_6 groups with no significant differences between the diseased groups. There was significant differences in the above parameters between the normal control groups and the patient groups. However, the tissue tonometry parameters became abnormal only from the C_{4b} group onwards suggesting that the tissue response to indentation with a loaded weight in assessing the mechanical properties of the skin is a significant factor in determining the groups that are most susceptible to venous ulceration.

This study showed that all patients with grades $C_3 - C_6$ venous disease had very similar measurements of venous physiology, whatever parameter was assessed. Interestingly, there was rather poor correlation between the APG measures and duplex assessments. It became apparent that duplex ultrasonography is good at identifying the presence or absence of venous reflux, but is poor at its quantification. This technique is best used as

an anatomical test of the presence or absence of reflux in particular veins. APG provides an overall impression of the extent of venous reflux, but did not identify those patients with the more severe stages of venous disease as having more severe venous reflux or calf muscle pump impairment. This could have been due to patient selection in this study, this is, only patients fit enough to perform the APG tests were included and these may have had better venous function than the remaining patients who were considered unsuitable. Perhaps the clinical outcome is also dependent on how much of the time patients spend with their lower limbs elevated or how much exercise they take, factors which could not be considered in this study. However, it seems more likely that venous physiological abnormalities are only part of the picture in the development of skin changes. The response of the skin to venous hypertension probably varies considerably in different patients and this may be one of the main explanations for the failure of the parameters of venous physiology to predict which patients will develop skin damage. There was no correlation between the tissue tonometry measurement that so well defines the severity of LDS and any APG, PPG or duplex ultrasound parameter, reflecting the lack of a direct relationship between these factors.

4.2. Future plans

Ethics approval has been sought for a study investigating the role of plasma vascular endothelial growth factor (VEGF) as a marker of chronic venous disease on various parameters including tissue tonometry parameters as described in this thesis, microcirculatory parameters as defined by laser Doppler fluxmetry, duplex ultrasound derived parameters and clinical parameters as defined by the CEAP method.

This will study in great detail the relationship between the clinical features of venous disease assessed by tonometry and the pathological factors known to be involved in venous skin damage, such as VEGF and other endothelial adhesion molecules.

SECTION V

Appendices

Appendix I

Presentations to learned societies

Objective assessment of skin changes in venous disease using a tissue tonometer.

J Farrah, M Saharay, J Scurr and PD Coleridge Smith.

Presented at the 83rd meeting of the Surgical Research Society at Oxford University in January 1996.

Objective assessment of skin changes in venous disease using a tissue tonometer.

J Farrah, M Saharay, J Scurr and PD Coleridge Smith.

Presented at the 9th Annual congress of the North American Society of Phlebology in San Diego, California in February 1996.

Objective assessment of skin damage in Chronic Venous insufficiency.

PD Coleridge Smith, J Farrah, M Saharay and J Scurr.

Presented at the Deutscher Phlebologenkongress in Berlin, Germany in September 1996.

Assessment of the severity of venous disease: Comparison between CEAP classification and tissue tonometry.

J Farrah, M Saharay, J Scurr and PD Coleridge Smith.

Presented at the Venous forum of the Royal Society of Medicine in Manchester in October 1996.

Compression stockings improve skin compliance in patients with chronic venous disease of the lower limbs.

J Farrah, M Saharay, J Scurr and PD Coleridge Smith.

Presented at the 1st Anglo-German symposium on Venous disorders in Berlin, Germany in June 1997.

Future Presentations

Assessment of the intervariability and ease of use of the CEAP method of classification and scoring of lower limb venous disease between different examiners.

Farrah J, Saharay M, Lambourne B, Boden L, Scurr J, Coleridge Smith PD

Accepted for presentation at the XIII UIP World congress on Phlebology in Sydney, Australia in September 1998.

Objective assessment of skin changes in venous disease using a tissue tonometer.

Farrah J, Saharay M, Scurr J, Coleridge-Smith PD

Accepted for presentation at the XIII UIP World congress on Phlebology in Sydney, Australia in September 1998.

Assessment of skin compliance in patients with chronic venous disease of the lower limb after compression stocking wear.

Farrah J, Saharay M, Scurr J, Coleridge-Smith P.

Accepted for presentation at the XIII UIP World congress on Phlebology in Sydney, Australia in September 1998.

Appendix II
Ethics approval and consent forms

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Mr P Coleridge Smith
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Vascular Laboratory
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02 December 1996

Dear Mr Coleridge Smith

Study No: 96/3513 *(Please quote in all correspondence)*
Title: **Correlation of the clinical classification of lower limb venous disease with standard non-invasive haemodynamic laboratory measurements and the objective assessment of the outcome of treatment of lower limb venous disease**

I am writing to let you know that I have looked at the above project and have given it Chairman's Approval. You may therefore go ahead with your study.

Please note that it is important that you notify the Committee of any adverse events or changes (name of investigator etc) relating to this project. You should also notify the Committee on completion of the project or indeed if the project is abandoned. Please remember to quote the above number in any correspondence.

Yours sincerely

Andre McLean
Chairman

The University College London Hospitals

CONFIDENTIAL

Department of Surgery

University College London Medical School

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E-mail: p.coleridgesmith@ucl.ac.uk

VOLUNTEERS REQUIRED

Subject Information Sheet

Volunteers are invited to participate in a study of the mechanisms of blood flow in their legs.

We are interested in the things which cause varicose veins and ulcers.

We are trying to compare the mechanisms of blood flow in normal subjects with no known or obvious circulatory problems to those who have varicose veins with or without ulcers.

The information we need from you is very simple.
Ultrasound scanning is used to test the veins in the leg.
We will use an air bag which is placed around the calf to measure changes in leg volume lying down and standing and also when performing simple tip-toe exercises.
We will also examine the skin in the area above your ankle using a special measuring device called a tonometer.
You will only need to make one visit to the Vascular Laboratory at the Middlesex Hospital for a period of about 2 hours.
No pain or needles are involved in this study.
You do not have to take part in this study if you do not want to. If you decide to take part you may withdraw at any time without having to give a reason.
All data obtained will be treated as strictly confidential.

If you have any other queries or questions regarding this study, please do not hesitate to ask.

You can call John Farrah in the Vascular Laboratory on

0171 380 9414

Mr J Farrah
Accredited Vascular Technologist

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An investigation of the mechanisms of blood flow in normal subjects.

Subject Consent form

1. Have you read the information sheet about this study YES/NO
2. Have you had an opportunity to ask questions and discuss this study? YES/NO
3. Have you received satisfactory answers to all your questions YES/NO
4. Have you received enough information about this study? YES/NO
5. Which doctor have you spoken to about this study? Dr.....
6. Do you understand that you are free to withdraw from this study
   - at any time
   - without giving a reason for withdrawing

7 Do you agree to take part in this study? YES/NO

Signed..... Date:.....

Name in Block Letters:.....

Doctor.....

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VOLUNTEERS REQUIRED

An investigation of the mechanisms of blood flow in patients with venous disease.

Patient Information Sheet

Volunteers are invited to participate in a study of the mechanisms of blood flow in their legs.
We are interested in the things which cause varicose veins and leg ulcers.
The information we need from you is very simple.
Ultrasound scanning is used to test the veins in the leg.
We will use an air bag which is placed around the calf to measure changes in leg volume lying down and standing and also when performing simple tip-toe exercises.
We will also examine the skin in the area above your ankle using a special measuring device called a tonometer.
You will only need to make one visit to the Vascular Laboratory at the Middlesex Hospital for a period of about 2 hours.
No pain or needles are involved in this study.
You do not have to take part in this study if you do not want to. If you decide to take part you may withdraw at any time without having to give a reason.
Your decision whether to take part or not will not affect your care and management in any way.
All data obtained will be treated as strictly confidential.

If you have any other queries or questions regarding this study, please do not hesitate to ask.

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An investigation of the mechanisms of blood flow in patients with venous disease.

Patient Consent form

1. Have you read the information sheet about this study YES/NO

2. Have you had an opportunity to ask questions and discuss this study? YES/NO

3. Have you received satisfactory answers to all your questions YES/NO

4. Have you received enough information about this study? YES/NO

5. Which doctor have you spoken to about this study? Dr.....

6. Do you understand that you are free to withdraw from this study

- at any time
- without giving a reason for withdrawing
- without affecting your future medical care?

7 Do you agree to take part in this study? YES/NO

Signed..... Date:.....

Name in Block Letters:.....

Doctor.....

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VOLUNTEERS REQUIRED

An investigation of the effects of compression stockings in patients with venous disease.

Patient Information Sheet

Volunteers are invited to participate in a study to assess the effects of wearing compression stockings prescribed by their Vascular Surgeons.

The information we need from you is very simple.

You have already had ultrasound scanning to test the veins in your legs and based on this report the Vascular Surgeon has decided that wearing compression stockings will help to improve the condition of your leg.

We need to make two measurements on your legs to assess the benefits of wearing the stockings:

to examine the area of skin above your ankle using a special measuring device called a tonometer, and to measure the volume of your leg using a tape measure.

You will only need to make two visits to the Vascular Laboratory at the Middlesex Hospital, once before the compression stockings are prescribed and then 4 weeks later after wearing the stockings. The tests will take about 30 minutes on each visit.

No pain or needles are involved in this study.

You do not have to take part in this study if you do not want to. If you decide to take part you may withdraw at any time without having to give a reason.

Your decision whether to take part or not will not affect your care and management in any way.

All data obtained will be treated as strictly confidential.

If you have any other queries or questions regarding this study, please do not hesitate to ask.

You can **call John Farrah** in the Vascular Laboratory on

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An investigation of the effects of compression stockings in patients with venous disease.

Patient Consent form

1. Have you read the information sheet about this study YES/NO

2. Have you had an opportunity to ask questions and discuss this study? YES/NO

3. Have you received satisfactory answers to all your questions YES/NO

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5. Which doctor have you spoken to about this study? Dr.....

6. Do you understand that you are free to withdraw from this study

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- without giving a reason for withdrawing
- without affecting your future medical care?

7 Do you agree to take part in this study? YES/NO

Signed..... Date:.....

Name in Block Letters:.....

Doctor.....

Appendix III

Study I

TONOMETER REPRODUCIBILITY STUDY (normal controls)						
		Phase I	Phase II		Phase III	
Subject	Visits	X_O	X_I-X_O	-1/Tau	X_I-X_O	-1/Tau
1	Day 1	3.10	0.27	2.77	0.11	630.00
1	Day 2	2.74	0.31	2.50	0.20	52.50
1	Day 3	3.00	0.27	2.53	0.01	730.50
	STDEV	0.19	0.02	0.15	0.10	365.90
	Average(mean)	2.95	0.28	2.60	0.11	471.00
	COV(%)	6.31	8.15	5.69	89.10	77.69
	Confidence Interval	0.21	0.03	0.17	0.11	414.04
	From	2.74	0.26	2.43	0.00	56.96
	to	3.16	0.31	2.77	0.21	885.04
2	Day 1	2.41	0.39	2.42	0.06	62.80
2	Day 2	2.47	0.45	2.40	0.26	54.60
2	Day 3	2.28	0.36	3.14	0.14	46.20
	STDEV	0.10	0.05	0.42	0.10	8.30
	Average(mean)	2.39	0.40	2.65	0.15	54.53
	COV(%)	4.07	11.46	15.89	65.65	15.22
	Confidence Interval	0.11	0.05	0.48	0.11	9.39
	From	2.28	0.35	2.18	0.04	45.14
	to	2.50	0.45	3.13	0.27	63.93
3	Day 1	2.08	0.31	4.88	0.10	885.10
3	Day 2	2.34	0.18	2.45	0.02	133.50
3	Day 3	2.31	0.42	2.14	0.09	80.60
	STDEV	0.14	0.12	1.50	0.04	449.99
	Average(mean)	2.24	0.30	3.16	0.07	366.40
	COV(%)	6.34	39.61	47.53	62.27	122.81
	Confidence Interval	0.16	0.14	1.70	0.05	509.20
	From	2.08	0.17	1.46	0.02	-142.80
	to	2.40	0.44	4.85	0.12	875.60
4	Day 1	2.28	0.33	3.62	0.23	324.50
4	Day 2	2.31	0.31	2.69	0.20	94.40
4	Day 3	2.24	0.29	2.85	0.01	441.40
	STDEV	0.03	0.02	0.50	0.12	176.55
	Average(mean)	2.28	0.31	3.05	0.15	286.77
	COV(%)	1.45	6.45	16.28	81.34	61.57
	Confidence Interval	0.04	0.02	0.56	0.14	199.78
	From	2.24	0.29	2.49	0.01	86.98
	to	2.32	0.33	3.62	0.28	486.55
5	Day 1	2.21	0.82	4.37	0.03	289.60
5	Day 2	2.31	1.31	1.91	0.03	289.60
5	Day 3	2.64	0.41	1.51	0.01	106.20
	STDEV	0.23	0.45	1.55	0.01	105.89
	Average(mean)	2.39	0.85	2.60	0.02	228.47
	COV(%)	9.43	53.22	59.64	49.49	46.35
	Confidence Interval	0.25	0.51	1.75	0.01	119.82
	From	2.13	0.34	0.84	0.01	108.65
	to	2.64	1.36	4.35	0.04	348.29

SAME DAY REPEATABILITY STUDY (30 minute intervals between measurements)						
		Phase I	Phase II		Phase III	
Subject	One visit	X_O	X_I-X_O	-1/Tau	X_I-X_O	-1/Tau
1	Trace 1	5.2	0.5	3.0	0.1	61.2
1	Trace 2	5.5	0.5	1.9	0.2	79.8
1	Trace 3	5.2	0.6	3.7	0.1	71.2
1	Trace 4	5.6	0.3	3.8	0.1	73.3
	STDEV	0.2	0.1	0.9	0.0	7.7
	Average(mean)	5.4	0.5	3.1	0.1	71.4
	COV(%)	4.5	22.1	28.8	30.8	10.8
	Confidence Interval	0.3	0.1	1.0	0.0	8.7
	From	5.1	0.4	2.1	0.1	62.7
	to	5.6	0.6	4.1	0.2	80.1

TONOMETER REPRODUCIBILITY STUDY (patients)						
		Phase I	Phase II		Phase III	
Subject	Visit	X_O	X_I-X_O	-1/Tau	X_I-X_O	-1/Tau
1	1	1.0	0.2	3.4	0.1	70.1
1	2	1.0	0.4	3.4	0.8	103.7
1	3	0.9	0.3	2.2	0.1	228.8
	STDEV	0.0	0.1	0.7	0.4	83.6
	Average(mean)	1.0	0.3	3.0	0.3	134.2
	COV(%)	3.1	25.9	22.1	121.7	62.3
	Confidence Interval	0.0	0.1	0.8	0.4	94.6
	From	0.9	0.2	2.3	-0.1	39.6
	to	1.0	0.4	3.8	0.8	228.8
2	1.0	2.9	0.6	5.1	0.8	77.6
2	2.0	3.0	0.5	2.4	0.2	52.4
2	3.0	3.0	0.4	2.3	0.1	119.7
	STDEV	0.1	0.1	1.6	0.4	34.0
	Average(mean)	3.0	0.5	3.3	0.3	83.2
	COV(%)	1.7	18.0	49.7	106.7	40.9
	Confidence Interval	0.1	0.1	1.8	0.4	38.5
	From	2.9	0.4	1.4	-0.1	44.8
	to	3.0	0.6	5.1	0.8	121.7
3	1	2.7	0.4	2.9	0.7	65.5
3	2	2.5	0.4	1.5	0.3	44.4
3	3	2.7	0.5	3.6	0.4	97.0
	STDEV	0.1	0.0	1.1	0.2	26.5
	Average(mean)	2.7	0.4	2.6	0.5	69.0
	COV(%)	5.0	11.3	41.0	44.5	38.4
	Confidence Interval	0.2	0.1	1.2	0.2	30.0
	From	2.5	0.4	1.4	0.2	39.0
	to	2.8	0.5	3.8	0.7	98.9

Calculating overall % COV in tissue tonometry parameters in normal controls						
Step 1						
Take Standard Deviation of each set of observations and square it to get variance.						
Phase I	Phase II			Phase III		
X_O	X_I-X_O	-1/Tau		X_I-X_O	-1/Tau	
0.04	0.00	0.85		0.00	86.65	
0.03	0.00	0.02		0.01	133881.75	
0.01	0.00	0.18		0 01	68.89	
0.02	0.01	2.25		0.00	202486.87	
0.05	0.20	2.40		0 00	11211.85	
Step 2						
Add all squares up						
Phase I	Phase II			Phase III		
X_O	X_I-X_O	-1/Tau		X_I-X_O	-1/Tau	
0.16	0.22	5.95		0.04	378906.12	
Step3						
Divide squares by no of observations (x3).						
Phase I	Phase II			Phase III		
X_O	X_I-X_O	-1/Tau		X_I-X_O	-1/Tau	
0.05	0.07	1.98		0 01	126302.04	
Step 4						
Take square root to get Standard Deviation						
Phase I	Phase II			Phase III		
X_O	X_I-X_O	-1/Tau		X_I-X_O	-1/Tau	
0.23	0.27	1.41		0.11	355.39	
Step 5						
Calculate grand mean of all observations						
Phase I	Phase II			Phase III		
X_O	X_I-X_O	-1/Tau		X_I-X_O	-1/Tau	
2.92	0.44	2.82		0.10	246.32	
Step 6						
Calculate ratio of SD/grand mean to get overall COV						
Phase I	Phase II			Phase III		
X_O	X_I-X_O	-1/Tau		X_I-X_O	-1/Tau	
0.08	0.61	0.50		1.08	1.44	
Overall COV (%) for each tonometry parameter in normal controls						
Phase I	Phase II			Phase III		
X_O	X_I-X_O	-1/Tau		X_I-X_O	-1/Tau	
COV(%) 8.0	61.5	50.0		108.2	144.3	

	Calculating overall % COV in tissue tonometry parameters in patients.				
	Step 1				
	Take SD of each set of observations and square it to get variance.				
	Phase I	**Phase II**		**Phase III**	
	X_O	**X_I-X_O**	**-1/Tau**	**X_I-X_O**	**-1/Tau**
	0.00	0.01	0.44	0.15	6994.11
	0.00	0.01	2.62	0.13	1156.12
	0.02	0.00	1.15	0.05	700.70
	Step 2				
	Add all squares up				
	Phase I	**Phase II**		**Phase III**	
	X_O	**X_I-X_O**	**-1/Tau**	**X_I-X_O**	**-1/Tau**
	0.02	0.02	4.21	0.33	8850.94
	Step3				
	Divide squares by no of observations (x3).				
	Phase I	Phase II		Phase III	
	X_O	**X_I-X_O**	**-1/Tau**	**X_I-X_O**	**-1/Tau**
	0.01	**0.01**	**1.40**	**0.11**	**2950.31**
	Step 4				
	Take square root to get SD				
	Phase I	**Phase II**		**Phase III**	
	X_O	**X_I-X_O**	**-1/Tau**	**X_I-X_O**	**-1/Tau**
	0.08	0.08	1.18	0.33	54.32
	Step 5				
	Calculate grand mean of all observations				
	Phase I	**Phase II**		**Phase III**	
	X_O	**X_I-X_O**	**-1/Tau**	**X_I-X_O**	**-1/Tau**
	2.20	0.43	2.96	0.38	95.47
	Step 6				
	Calculate ratio of SD/grand mean to get overall COV				
	Phase I	**Phase II**		**Phase III**	
	X_O	**X_I-X_O**	**-1/Tau**	**X_I-X_O**	**-1/Tau**
	0.04	0.19	0.40	0.86	0.57
	Overall COV (%) for each tonometry parameter in patients				
	Phase I	**Phase II**		**Phase III**	
	X_O	**X_I-X_O**	**-1/Tau**	**X_I-X_O**	**-1/Tau**
COV(%)	**3.81**	**18.52**	**40.02**	**86.49**	**56.90**

Pre- and post-measurement calibration check for the tissue tonometer		
Subject No	**Pre-**	**Post-**
1	40.1	40.3
2	39.7	39.5
3	42.4	42.1
4	42.8	42.8
5	41.2	41.1
6	32.6	32
7	28.8	29
8	32.1	32.5
9	31.2	31.3
10	31.5	31.9
11	43.3	43.9
12	41.7	41.5
13	28.5	28.3
14	27.5	27
15	29	29.2
16	29.3	29.3
17	39.9	40.1
18	42.6	42.3
19	28.5	28.1
20	29	29.1
21	31.5	31.3
22	40.3	40.1
23	29	29
24	32	32
25	41.4	41.2
26	29.1	29
27	41	41.3
28	40.6	41
29	28.2	28.5
30	28	28

Tissue tonometry distance and rate constant parameters between young and old controls					
	Distance moved X_0 (mm) during phase I			Distance moved X_I-X_0 (mm)during phase II	
	normal young	normal old		normal young	normal old
	1.8	3.2		0.3	0.3
	2.6	2.4		0.4	0.2
	2.3	4.5		0.3	0.4
	3.1	3.0		0.3	0.3
	2.3	4.0		0.4	0.3
	2.4	3.5		0.4	0.5
	2.8	3.1		0.0	0.3
	2.4	2.9		0.2	0.5
	4.1	2.0		0.2	0.4
	1.6	2.4		1.3	0.1
	2.6	2.1		0.4	0.1
	3.1	1.4		0.4	0.1
	1.9	2.0		0.2	0.1
	3.6	1.1		0.3	0.2
	2.5	2.9		0.2	0.2
	3.2	3.8		0.3	0.8
	2.8	2.6		0.3	0.1
	3.6	2.9		0.3	0.4
	3.5	2.9		0.3	0.2
	2.7	3.7		0.2	0.2
	3.0	3.3		0.2	0.3
median	2.7	2.9	median	0.3	0.3
LQ	2.4	2.4	LQ	0.2	0.2
UQ	3.1	3.3	UQ	0.4	0.4

	Rate constant parameter (-1/Tau in $secs^{-1}$) during phase			Distance moved X_I-X_0 (mm)during phase III	
	normal young	normal old		normal young	normal old
	3.0	2.2		0.1	0.1
	4.2	2.4		0.6	0.1
	3.6	1.6		0.0	0.1
	2.8	2.1		0.1	0.0
	2.1	3.2		0.1	0.1
	2.4	2.1		0.1	0.8
	5.8	2.4		0.1	0.0
	2.0	1.6		0.0	0.3
	2.6	2.5		0.2	0.1
	1.6	3.1		0.2	0.0
	1.8	3.6		0.0	0.0
	2.3	2.7		0.2	0.0
	4.9	2.2		0.3	0.1
	3.3	3.1		0.3	0.2
	4.4	1.7		0.1	0.1
	3.6	1.7		0.3	0.0
	2.4	2.5		0.2	0.1
	2.6	2.4		0.0	0.0
	2.6	1.7		0.0	0.0
	3.6	2.4		0.2	0.0
	2.6	4.5		0.1	0.0
median	2.6	2.4	media	0.1	0.1
LQ	2.4	2.1	LQ	0.1	0.0
UQ	3.6	2.7	UQ	0.2	0.1

	Rate constant parameter (-1/Tau in $secs^{-1}$) during phase III	
	normal young	normal old
	60.4	58.0
	158.5	40.8
	155.1	86.8
	701.5	1946.8
	861.8	67.7
	62.8	45.2
	1064.5	149.0
	75.9	259.9
	290.2	41.0
	99.2	149.7
	112.4	44.6
	44.9	116.9
	486.8	51.6
	315.8	798.0
	68.6	3114.0
	67.1	379.3
	82.5	842.4
	94.0	341.7
	90.2	436.8
	146.8	84.4
	59.0	119.5
median	99.2	119.5
LQ	68.6	58.0
UQ	290.2	379.3

Distance and rate constant parameters between normal (C_0) and patients (C_3, C_{4a} and C_{4b})

Distance travelled (X_t-X_0 in mm) during phase I							
C_0	1.8	C_3	2.6	C_{4a}	2.0	C_{4b}	1.2
C_0	2.6	C_3	3.2	C_{4a}	2.4	C_{4b}	1.7
C_0	2.3	C_3	1.9	C_{4a}	2.0	C_{4b}	0.6
C_0	3.1	C_3	2.9	C_{4a}	3.2	C_{4b}	0.6
C_0	2.3	C_3	1.4	C_{4a}	2.8	C_{4b}	0.7
C_0	2.4	C_3	2.3	C_{4a}	3.8	C_{4b}	0.5
C_0	2.8	C_3	1.6	C_{4a}	2.2	C_{4b}	1.1
C_0	2.4	C_3	1.1	C_{4a}	2.3	C_{4b}	0.4
C_0	4.1	C_3	1.1	C_{4a}	3.4	C_{4b}	0.9
C_0	1.6	C_3	3.1	C_{4a}	2.9	C_{4b}	1.1
C_0	3.2	C_3	3.5	C_{4a}	2.2	C_{4b}	0.6
C_0	2.4	C_3	4.2	C_{4a}	2.8	C_{4b}	1.2
C_0	2.6	C_3	3.1	C_{4a}	1.8	C_{4b}	0.9
C_0	4.5	C_3	2.2	C_{4a}	1.8	C_{4b}	1.1
C_0	3.0	C_3	1.5	**median**	**2.3**	C_{4b}	0.4
C_0	4.0	C_3	1.5	**LO**	**2.0**	C_{4b}	0.9
C_0	3.5	C_3	1.2	**UO**	**2.9**	C_{4b}	1.0
C_0	3.1	C_3	1.5			C_{4b}	1.3
C_0	2.9	C_3	3.0			C_{4b}	1.1
C_0	2.0	C_3	2.8			C_{4b}	0.3
C_0	2.4	C_3	2.0			C_{4b}	1.2
C_0	2.1	C_3	3.0			C_{4b}	0.5
C_0	3.1	C_3	3.5			C_{4b}	0.7
C_0	1.4	C_3	2.2			C_{4b}	0.8
C_0	1.9	C_3	3.8			C_{4b}	1.5
C_0	2.0	C_3	1.7			C_{4b}	0.8
C_0	1.1	C_3	2.9			C_{4b}	1.5
C_0	2.9	C_3	3.3			C_{4b}	0.8
C_0	3.8	**median**	**2.7**			C_{4b}	1.6
C_0	2.6	**LO**	**1.6**			C_{4b}	0.9
C_0	3.6	**UO**	**3.1**			C_{4b}	0.7
C_0	2.4					C_{4b}	0.7
C_0	3.3					C_{4b}	0.9
C_0	3.3					C_{4b}	0.6
C_0	2.8					C_{4b}	0.8
C_0	2.9					C_{4b}	0.9
C_0	2.9					C_{4b}	0.9
C_0	3.7					C_{4b}	1.3
C_0	3.6					C_{4b}	0.6
C_0	3.5					C_{4b}	0.9
C_0	2.7					C_{4b}	0.5
C_0	3.0					C_{4b}	0.7
median	**2.9**					C_{4b}	1.2
LO	**2.4**					C_{4b}	1.2
UO	**3.3**					**median**	**0.8**
						LO	**0.7**
						UO	**1.2**

Distance travelled (X_t-X_0 in mm) during phase II							
C_0	0.3	C_3	0.1	C_{4a}	0.4	C_{4b}	0.5
C_0	0.4	C_3	0.6	C_{4a}	0.5	C_{4b}	0.6
C_0	0.3	C_3	0.4	C_{4a}	0.9	C_{4b}	0.2
C_0	0.3	C_3	0.9	C_{4a}	0.4	C_{4b}	0.6
C_0	0.4	C_3	0.5	C_{4a}	0.7	C_{4b}	0.7
C_0	0.4	C_3	0.5	C_{4a}	0.9	C_{4b}	0.6
C_0	0.0	C_3	0.8	C_{4a}	0.8	C_{4b}	1.1
C_0	0.2	C_3	1.7	C_{4a}	0.9	C_{4b}	0.3
C_0	0.2	C_3	0.7	C_{4a}	0.9	C_{4b}	0.4
C_0	1.3	C_3	0.7	C_{4a}	0.5	C_{4b}	0.6
C_0	0.3	C_3	0.5	C_{4a}	0.6	C_{4b}	0.6
C_0	0.2	C_3	0.6	C_{4a}	1.0	C_{4b}	1.1
C_0	0.4	C_3	1.2	C_{4a}	1.0	C_{4b}	0.8
C_0	0.4	C_3	0.2	C_{4a}	0.5	C_{4b}	0.5
C_0	0.3	C_3	0.2	**median**	**0.7**	C_{4b}	0.2
C_0	0.3	C_3	0.2	**LO**	**0.5**	C_{4b}	0.8
C_0	0.5	C_3	1.3	**UO**	**0.9**	C_{4b}	0.4
C_0	0.3	C_3	0.4			C_{4b}	0.6
C_0	0.5	C_3	0.5			C_{4b}	1.1
C_0	0.4	C_3	0.7			C_{4b}	0.2
C_0	0.1	C_3	0.4			C_{4b}	0.0
C_0	0.1	C_3	0.5			C_{4b}	0.5
C_0	0.4	C_3	1.0			C_{4b}	0.7
C_0	0.1	C_3	0.4			C_{4b}	0.2
C_0	0.2	C_3	0.7			C_{4b}	0.4
C_0	0.1	C_3	1.0			C_{4b}	0.8
C_0	0.2	C_3	0.6			C_{4b}	0.4
C_0	0.2	C_3	0.3			C_{4b}	0.8
C_0	0.8	**median**	**0.6**			C_{4b}	0.3

C_0	0.1	LO	0.4			C_{4b}	0.3
C_0	0.3	UO	0.7			C_{4b}	0.1
C_0	0.2					C_{4b}	0.8
C_0	0.3					C_{4b}	0.8
C_0	0.3					C_{4b}	0.4
C_0	0.3					C_{4b}	0.2
C_0	0.4					C_{4b}	0.5
C_0	0.2					C_{4b}	0.4
C_0	0.2					C_{4b}	0.2
C_0	0.3					C_{4b}	1.0
C_0	0.3					C_{4b}	0.1
C_0	0.2					C_{4b}	0.3
C_0	0.2					C_{4b}	0.6
median	0.3					C_{4b}	0.6
LO	0.2					median	0.5
UO	0.4					LO	0.3
						UO	0.7

Rate constant parameter (-1/Tau in secs $^{-1}$) during phase

C_0	3.0	C_3	3.0	C_{4a}	4.6	C_{4b}	3.8
C_0	4.2	C_3	1.8	C_{4a}	2.4	C_{4b}	2.4
C_0	3.6	C_3	3.6	C_{4a}	4.6	C_{4b}	2.8
C_0	2.8	C_3	1.9	C_{4a}	3.4	C_{4b}	3.7
C_0	2.1	C_3	4.0	C_{4a}	4.4	C_{4b}	2.1
C_0	2.4	C_3	11.1	C_{4a}	2.6	C_{4b}	2.4
C_0	5.8	C_3	7.6	C_{4a}	7.0	C_{4b}	3.2
C_0	2.0	C_3	2.3	C_{4a}	2.2	C_{4b}	4.6
C_0	2.6	C_3	3.6	C_{4a}	2.3	C_{4b}	3.0
C_0	1.6	C_3	2.6	C_{4a}	2.6	C_{4b}	4.3
C_0	2.2	C_3	2.7	C_{4a}	3.8	C_{4b}	2.6
C_0	2.4	C_3	2.2	C_{4a}	1.6	C_{4b}	2.7
C_0	1.8	C_3	4.4	C_{4a}	3.3	C_{4b}	2.4
C_0	1.6	C_3	3.1	C_{4a}	4.4	C_{4b}	4.1
C_0	2.1	C_3	2.1	median	3.3	C_{4b}	3.6
C_0	3.2	C_3	1.8	LO	2.4	C_{4b}	2.6
C_0	2.1	C_3	2.8	UO	4.4	C_{4b}	2.4
C_0	2.4	C_3	2.7			C_{4b}	9.0
C_0	1.6	C_3	6.3			C_{4b}	3.2
C_0	2.5	C_3	4.0			C_{4b}	2.7
C_0	3.1	C_3	2.5			C_{4b}	4.6
C_0	3.6	C_3	4.6			C_{4b}	4.1
C_0	2.3	C_3	5.1			C_{4b}	2.6
C_0	2.7	C_3	4.0			C_{4b}	2.4
C_0	4.9	C_3	4.0			C_{4b}	2.2
C_0	2.2	C_3	4.1			C_{4b}	2.6
C_0	3.1	C_3	2.6			C_{4b}	3.1
C_0	1.7	C_3	4.7			C_{4b}	2.2
C_0	1.7	median	3.3			C_{4b}	2.1
C_0	2.5	LO	2.6			C_{4b}	2.2
C_0	3.3	UO	4.2			C_{4b}	4.2
C_0	4.4					C_{4b}	5.7
C_0	4.5					C_{4b}	2.0
C_0	3.6					C_{4b}	2.5
C_0	2.4					C_{4b}	2.8
C_0	2.4					C_{4b}	3.8
C_0	1.7					C_{4b}	3.3
C_0	2.4					C_{4b}	2.5
C_0	2.6					C_{4b}	2.0
C_0	2.6					C_{4b}	7.2
C_0	3.6					C_{4b}	1.8
C_0	2.6					C_{4b}	4.8
median	2.5					C_{4b}	5.8
LO	2.1					median	2.8
UO	3.2					LO	2.4
						UO	3.9

Distance travelled (X_1-X_0 in mm) during phase III

C_0	0.1	C_3	0.1	C_{4a}	0.5	C_{4b}	0.5
C_0	0.6	C_3	0.3	C_{4a}	0.2	C_{4b}	0.7
C_0	0.0	C_3	0.1	C_{4a}	0.7	C_{4b}	0.0
C_0	0.1	C_3	0.8	C_{4a}	0.3	C_{4b}	0.2
C_0	0.1	C_3	0.6	C_{4a}	0.8	C_{4b}	0.4
C_0	0.1	C_3	0.3	C_{4a}	0.7	C_{4b}	0.2
C_0	0.1	C_3	0.9	C_{4a}	0.9	C_{4b}	0.4
C_0	0.0	C_3	2.3	C_{4a}	0.2	C_{4b}	0.1
C_0	0.2	C_3	0.3	C_{4a}	0.0	C_{4b}	0.1
C_0	0.2	C_3	0.6	C_{4a}	0.3	C_{4b}	0.5
C_0	0.1	C_3	0.0	C_{4a}	0.3	C_{4b}	0.4
C_0	0.1	C_3	0.1	C_{4a}	0.5	C_{4b}	0.9
C_0	0.0	C_3	1.7	C_{4a}	0.6	C_{4b}	0.5
C_0	0.1	C_3	0.8	C_{4a}	0.5	C_{4b}	0.7
C_0	0.0	C_3	0.1	median	0.5	C_{4b}	0.1

C_0	0.1	C_3	2.7	LO	0.3	C_{4b}	0 1
C_0	0 8	C_3	0 0	UO	0.7	C_{4b}	0.2
C_0	0.0	C_3	0.8			C_{4b}	0.6
C_0	0 3	C_3	0.7			C_{4b}	0 4
C_0	0.1	C_3	0.5			C_{4b}	0.3
C_0	0 0	C_3	1.0			C_{4b}	0.3
C_0	0.0	C_3	0.1			C_{4b}	0.5
C_0	0 2	C_3	0.8			C_{4b}	0 6
C_0	0 0	C_3	0.9			C_{4b}	0 0
C_0	0 3	C_3	1.6			C_{4b}	0.5
C_0	0.1	C_3	0.4			C_{4b}	0.2
C_0	0.2	C_3	0.7			C_{4b}	0.2
C_0	0.1	median	0.6			C_{4b}	0.4
C_0	0 0	LO	0.2			C_{4b}	0 0
C_0	0.1	UO	0.9			C_{4b}	0.0
C_0	0.3					C_{4b}	0.0
C_0	0 1					C_{4b}	0 4
C_0	0.0					C_{4b}	0 8
C_0	0.3					C_{4b}	0.1
C_0	0 2					C_{4b}	0.0
C_0	0 0					C_{4b}	0.3
C_0	0 0					C_{4b}	0.2
C_0	0 0					C_{4b}	0.2
C_0	0 0					C_{4b}	0.0
C_0	0 0					C_{4b}	0.3
C_0	0.2					C_{4b}	0.5
C_0	0.1					C_{4b}	0.8
median	0 1					median	0.3
LO	0 0					LO	0.1
UO	0 2					UO	0.5

Rate constant parameter (-1/Tau in secs $^{-1}$) during phase

C_0	60.4	C_3	79 8	C_{4a}	20.4	C_{4b}	53.0
C_0	158.5	C_3	100.0	C_{4a}	56.6	C_{4b}	53.3
C_0	155.1	C_3	11.1	C_{4a}	56.1	C_{4b}	45.6
C_0	701.5	C_3	49.2	C_{4a}	62.5	C_{4b}	31.0
C_0	861 8	C_3	67.0	C_{4a}	56.1	C_{4b}	30.2
C_0	62.8	C_3	43.1	C_{4a}	47.7	C_{4b}	32.3
C_0	1064 5	C_3	73.1	C_{4a}	58.1	C_{4b}	38 8
C_0	75.9	C_3	53.1	C_{4a}	56.1	C_{4b}	46.5
C_0	290.2	C_3	54 5	C_{4a}	22.1	C_{4b}	27.8
C_0	99 2	C_3	41 6	C_{4a}	84.7	C_{4b}	56.6
C_0	58 0	C_3	131.3	C_{4a}	34 2	C_{4b}	28 5
C_0	40 8	C_3	72.7	C_{4a}	46 6	C_{4b}	78.6
C_0	112 4	C_3	44 6	C_{4a}	62.5	C_{4b}	53.9
C_0	86 8	C_3	21.3	C_{4a}	46.9	C_{4b}	79.2
C_0	1946 8	C_3	64.1	median	56.1	C_{4b}	31.5
C_0	67.7	C_3	150 0	LO	46.7	C_{4b}	59.2
C_0	45.2	C_3	115.1	UO	57.7	C_{4b}	74.3
C_0	149 0	C_3	80.1			C_{4b}	68.2
C_0	259 9	C_3	89 8			C_{4b}	38 0
C_0	41 0	C_3	54.1			C_{4b}	108.9
C_0	149.7	C_3	54 0			C_{4b}	3.6
C_0	44 6	C_3	65.3			C_{4b}	71.7
C_0	44 9	C_3	161.4			C_{4b}	60.3
C_0	116 9	C_3	196 0			C_{4b}	10 5
C_0	486 8	C_3	57.7			C_{4b}	23.0
C_0	51 6	C_3	44 6			C_{4b}	60.3
C_0	798 0	C_3	375.2			C_{4b}	50 0
C_0	3114 0	median	65 3			C_{4b}	36.0
C_0	379.3	LO	51.2			C_{4b}	15.0
C_0	842.4	UO	94.9			C_{4b}	21.5
C_0	315 8					C_{4b}	12.9
C_0	68 6					C_{4b}	31.0
C_0	119 5					C_{4b}	32.6
C_0	67.1					C_{4b}	18.1
C_0	82.5					C_{4b}	74.2
C_0	341.7					C_{4b}	47.7
C_0	436 8					C_{4b}	49.2
C_0	84 4					C_{4b}	84.1
C_0	94 0					C_{4b}	853.8
C_0	90 2					C_{4b}	157.8
C_0	146 8					C_{4b}	55.7
C_0	59 0					C_{4b}	66.1
median	114 7					median	41.8
LO	67.3					LO	25.0
UO	335 2					UO	64.7

Appendix IV

Study II

	Phase I (0-1 secs)	Phase II (1-10 secs)	Phase II (1-10 secs)	Phase III (10-300 secs)	Phase III (10-300 secs)
Glycerol (%)	X_O (mm)	X_f-X_O (mm)	-1/Tau (secs $^{-1}$)	X_f-X_O (mm)	-1/Tau (secs $^{-1}$)
0	4.82	0.36	2.44	0.28	176.60
0	4.72	0.25	2.85	0.17	72.00
0	4.26	0.34	2.55	0.23	63.20
Average	**4.60**	**0.32**	**2.61**	**0.23**	**103.93**
St Dev	**0.30**	**0.06**	**0.21**	**0.05**	**63.08**
COV%	**6.51**	**19.23**	**8.12**	**23.84**	**60.70**
25	2.57	0.63	3.20	0.57	59.00
25	2.57	0.90	3.45	0.71	60.60
25	1.85	0.81	2.64	1.19	56.30
Average	**2.33**	**0.78**	**3.10**	**0.82**	**58.63**
St Dev	**0.42**	**0.14**	**0.41**	**0.33**	**2.17**
COV%	**17.97**	**17.71**	**13.39**	**39.70**	**3.71**
50	1.35	0.88	4.58	1.39	127.10
50	1.62	1.14	2.39	1.58	54.90
50	1.35	0.85	2.67	1.61	58.40
Average	**1.44**	**0.96**	**3.21**	**1.53**	**80.13**
St Dev	**0.15**	**0.16**	**1.19**	**0.12**	**40.71**
COV%	**10.58**	**16.57**	**37.09**	**7.89**	**50.81**
100	0.50	0.15	2.91	0.32	77.70
100	0.36	0.06	5.98	0.13	93.20
100	0.33	0.08	3.96	0.15	77.20
Average	**0.40**	**0.10**	**4.28**	**0.20**	**82.70**
St Dev	**0.09**	**0.05**	**1.56**	**0.11**	**9.10**
COV%	**22.05**	**50.47**	**36.43**	**53.74**	**11.00**

***In vitro* reproducibility test of tissue tonometry parameters of distance and rate constants using Glycerol diluted with water on an open cell sponge done three times at the same times on different days.**

	C_2 Spring constant	C_1 Spring constant	k Dashpot constant	C_1 spring constant	k Dashpot constant
Glycerol (%)	Phase I	Phase II	Phase II	Phase III	Phase III
0	0.68	9.21	22.47	11.72	2070.10
0	0.70	13.39	38.17	19.13	1377.53
0	0.77	9.61	24.50	14.09	890.52
Average	**0.72**	**10.74**	**28.38**	**14.98**	**1446.05**
St Dev	**0.05**	**2.31**	**8.54**	**3.78**	**592.77**
COV%	**6.74**	**21.50**	**30.08**	**25.26**	**40.99**
25	1.28	5.22	16.70	5.79	341.65
25	1.28	3.64	12.57	4.65	281.50
25	1.78	4.08	10.76	2.76	155.58
Average	**1.45**	**4.31**	**13.34**	**4.40**	**259.58**
St Dev	**0.29**	**0.81**	**3.04**	**1.53**	**94.95**
COV%	**20.06**	**18.86**	**22.80**	**34.74**	**36.58**
50	2.43	3.72	17.04	2.36	300.39
50	2.03	2.88	6.89	2.08	114.00
50	2.43	3.87	10.33	2.04	119.00
Average	**2.30**	**3.49**	**11.42**	**2.16**	**177.80**
St Dev	**0.23**	**0.53**	**5.16**	**0.18**	**106.20**
COV%	**9.97**	**15.21**	**45.20**	**8.24**	**59.73**
100	6.65	21.36	62.15	10.14	788.20
100	9.06	52.22	312.25	26.11	2433.28
100	9.97	43.37	171.74	21.68	1674.01
Average	**8.56**	**38.98**	**182.05**	**19.31**	**1631.83**
St Dev	**1.72**	**15.89**	**125.37**	**8.24**	**823.35**
COV%	**20.07**	**40.77**	**68.87**	**42.68**	**50.46**

***In vitro* reproducibility test of the Kelvin-Hooke parameters of springs and dashpots using Glycerol diluted with water on an open cell sponge done three times at the same times on different days.**

Appendix V

Study III

Limb No	CEAP Clinical Classification	Phase I X_0 (mm)	Phase II X_1-X_0 (mm)	Phase II -1/Tau (secs $^{-1}$)	Phase III X_1-X_0 (mm)	Phase III -1/Tau (secs $^{-1}$)
1	C_0	2.46	0.33	2.54	0.20	45.70
2	C_0	3.07	0.34	2.96	0.18	54.30
3	C_0	2.62	0.17	3.00	0 28	325.83
4	C_0	2.57	0 24	1.91	0.31	57.45
5	C_0	3.38	0.27	2 65	0.10	536.50
6	C_0	3.71	0.28	2.55	0.07	86.23
7	C_0	2.49	0.17	2.10	0.28	101.50
8	C_0	2.56	0.31	2.30	0.06	64.33
9	C_0	1.75	0.25	3 03	0.10	60.40
10	C_0	2.57	0.37	4.19	0 64	158.50
11	C_0	2.28	0.33	3.62	0 04	155.10
12	C_0	3.10	0.27	2.77	0.12	701.50
13	C_0	2.31	0 41	2.14	0.10	861.80
14	C_0	2.41	0 39	2.42	0.06	62.80
15	C_0	2.77	0 02	5.75	0.12	1064.50
16	C_0	2 44	0.17	2.02	0.01	75.90
17	C_0	1.58	1.25	1.55	0.19	99.20
18	C_0	3.23	0.27	2.19	0.12	58.00
19	C_0	2 38	0.18	2.39	0 08	40 80
20	C_0	2 64	0.37	1.77	0.01	112.40
	Median	**2.57**	**0.28**	**2.48**	**0.11**	**92.72**
	LO	**2.40**	**0.22**	**2.13**	**0.07**	**59.80**
	UO	**2.84**	**0.35**	**2.97**	**0.19**	**200.33**
1	C_1	3.56	0.57	2.28	0.33	59.40
2	C_1	2.81	0.44	2.23	0.01	495.90
3	C_1	2 63	0.31	2.85	0.12	82.93
4	C_1	2.52	0.34	2.06	0.22	67.87
5	C_1	2.31	0.33	2.06	0.17	211.60
6	C_1	3 52	0 26	2.99	0.31	68.80
7	C_1	3 56	0 62	2.70	0.18	161.53
8	C_1	2 32	0.12	1.82	0.01	93.35
9	C_1	4 40	0.17	3.13	0.13	903.47
10	C_1	4 16	0.13	5.09	0 07	1602.60
11	C_1	4 49	0.40	1.64	0.10	86 80
12	C_1	3 00	0 28	2.13	0.17	194.68
13	C_1	3 96	0 29	3.18	0 05	67.70
14	C_1	3 53	0.47	2.10	0.84	45.20
15	C_1	3 14	0.33	2.40	0.01	149.00
16	C_1	2 94	0 48	1.59	0.27	259.90
17	C_1	2 01	0.38	2.54	0.12	41.00
18	C_1	2 44	0.10	3.08	0.02	149.70
19	C_1	2 05	0.10	3.56	0 02	44 60
20	C_1	3 07	0.36	2.34	0.21	44.90
21	C_1	1.39	0.13	2.65	0.02	116.90
22	C_1	1 88	0 19	4 90	0.34	486 80
23	C_1	1.98	0 14	2.16	0.11	51.60
24	C_1	1.12	0.20	3 08	0.20	798.00
25	C_1	2 91	0.21	1.72	0 09	3114.00
26	C_1	3 33	0.32	4.47	0.01	119.50
	median	**2.93**	**0.30**	**2.47**	**0.12**	**118.20**
	LO	**2.31**	**0.18**	**2.11**	**0.03**	**67.74**
	UO	**3.53**	**0.38**	**3.08**	**0.21**	**247.83**
1	C_2	3 04	1.23	1.80	0.24	179.70
2	C_2	2 54	0 69	2.42	0.89	62.00
3	C_2	3 80	0 80	2.14	0.75	58.80
4	C_2	2 81	0 34	3 06	0.26	81.83
5	C_2	2.99	0.37	2.92	0.39	108.10
6	C_2	2 95	0.35	3.71	0.38	65 65
7	C_2	3.50	0.25	3.44	0.29	76.80
8	C_2	2 90	0.52	5.26	0.44	78.30
9	C_2	2 48	0 43	1.97	0.21	132.20
10	C_2	2 31	0 42	2.06	0.02	135 80
11	C_2	2.38	0.12	3 01	0.06	79 80
12	C_2	1 91	0 37	3 55	0.06	11.10
13	C_2	3 53	0.53	2 69	0.02	131.30
14	C_2	1 45	0.23	2.11	0.10	64.10
15	C_2	1.52	0.43	2.65	0.02	115.10
16	C_2	4 16	0.59	2.18	0.14	72.70
17	C_2	2 20	0.16	3 07	0.80	21.30
	median	**2.90**	**0.43**	**2.69**	**0.24**	**78.30**

	LO	**2.43**	**0.36**	**2.16**	**0.06**	**64.10**
	UO	**3.27**	**0.56**	**3.25**	**0.39**	**115.10**
1	C_3	2.51	0 26	1.90	0.45	70.80
2	C_3	3 60	0.45	3.30	0.53	175.20
3	C_3	2 61	0.67	3.90	0 68	89.90
4	C_3	2 06	0.45	4.13	0 40	108.20
5	C_3	2.89	0.63	2.66	0.46	89 63
6	C_3	3 41	0.51	3.00	0.33	240.00
7	C_3	2.34	0.20	6.36	0.50	42.60
8	C_3	2 81	0.55	4.20	0.50	101 00
9	C_3	2.54	0.62	2.68	0.32	71.07
10	C_3	3.55	0.76	4.73	0.04	88.87
11	C_3	3.45	0.52	2.94	0.25	81 63
12	C_3	3.20	0.62	1.77	0.29	100.00
13	C_3	2 90	0 85	1.92	0.81	49.20
14	C_3	1.35	0.54	4.01	0.55	67.00
15	C_3	2.31	0.48	11.10	0.28	43.10
16	C_3	1.55	0 82	7.63	0.90	73.10
17	C_3	1.06	1.66	2.29	2.29	53.10
18	C_3	1.09	0 65	3.57	0.34	54.50
19	C_3	3.14	0.72	2.62	0.57	41.60
20	C_3	3.10	1.16	4 44	1.73	44.60
21	C_3	1 48	0.22	1.78	error	error
22	C_3	1.22	1.29	2.79	2.70	150 00
23	C_3	1 98	0.38	2.52	0.46	54.10
24	C_3	3 00	0.45	4.61	1.01	54 00
25	C_3	3 83	0 66	3.97	0.92	196 00
	median	**2.61**	**0.62**	**3.30**	**0.50**	**72.08**
	LO	**1.98**	**0.45**	**2.62**	**0.33**	**53.78**
	UO	**3.14**	**0.72**	**4.20**	**0.75**	**100.25**
1	C4a	2.48	0.29	3.09	0 90	39.10
2	C4a	2.21	0 40	3 07	0.51	59.60
3	C4a	2 89	0 92	1.89	0.81	71.57
4	C4a	2.52	0 99	4.15	0.87	77.73
5	C4a	2.71	0 61	2.73	0.59	61 00
6	C4a	3 47	0.82	4.93	0.65	55.70
7	C4a	2.20	0.31	3.48	0.25	49.50
8	C4a	3 00	0 53	6.31	0.77	64.70
9	C4a	2 93	0.74	3.01	0.22	137 07
10	C4a	2 62	0 67	1.98	0.69	72.73
11	C4a	2 87	0.59	8.59	1.14	223.70
12	C4a	2.90	0 67	4.44	0.82	56.00
13	C4a	3 63	0 93	2.54	0 66	50.20
14	C4a	1.95	0 44	4 60	0.51	20.40
15	C4a	2 41	0.48	2.35	0.16	56 60
16	C4a	1 98	0 93	4 63	0.72	56.10
17	C4a	3 23	0 37	3.38	0.27	62.50
18	C4a	2 24	0 80	7.01	0.94	58.10
19	C4a	2 28	0 89	2.24	0.22	56 10
20	C4a	3.37	0.86	2.32	0.01	22.10
21	C4a	2 94	0.45	2.60	0.30	84.70
22	C4a	2.21	0.57	3 83	0.31	34.20
23	C4a	1.75	0.48	4.43	0.50	46.90
	median	**2.62**	**0.61**	**3.38**	**0.59**	**56.60**
	LO	**2.23**	**0.47**	**2.57**	**0.29**	**49.85**
	UO	**2.93**	**0.84**	**4.52**	**0.79**	**68.13**
1	C_{4b}	0 86	0 43	4 01	0.55	28.10
2	C_{4b}	0.79	0 62	1.97	0.11	69.50
3	C_{4b}	0.73	0.95	5 09	1.31	195.50
4	C_{4b}	1 02	0.71	2 28	0.51	144.10
5	C_{4b}	1.22	0 94	5.14	0 61	54.30
6	C_{4b}	1.53	1.16	2.18	0.65	49.90
7	C_{4b}	1.45	0.92	3.33	0.77	42.40
8	C_{4b}	0 66	0 38	1.96	0.18	153.75
9	C_{4b}	0 89	0.74	3.75	0.12	58 50
10	C_{4b}	1.12	0.73	5.17	0.13	54.70
11	C_{4b}	1.15	0 45	3 81	0.51	53 0
12	C_{4b}	1.72	0.57	2.42	0.69	53.3
13	C_{4b}	0 59	0.20	2.80	0.03	45 60
14	C_{4b}	0.56	0.55	3 69	0.19	31.00
15	C_{4b}	0.73	0.70	2.13	0.42	30.2
16	C_{4b}	0 46	0.58	2.39	0.21	32.3
17	C_{4b}	1 09	1.05	3.23	0.37	38 8
18	C_{4b}	0 43	0 29	4 64	0 09	46 5
19	C_{4b}	0 56	0.56	2.55	0.37	28.5
20	C_{4b}	1 19	1 05	2.66	0.91	78 6
21	C_{4b}	0 82	0 40	2.48	0.10	18 10

22	C_{4b}	0.92	0 22	2.77	0.03	74.2
23	C_{4b}	0.86	0.45	3.77	0.28	47.7
24	C_{4b}	1.25	0.44	3.33	0.23	49.20
25	C_{4b}	0.56	0.16	2.53	tono fell off!!	
26	C_{4b}	1.15	0.59	4.82	0.50	55.70
	median	**0.96**	**0.67**	**3.33**	**0.23**	**54.30**
	LO	**0.80**	**0.43**	**2.33**	**0.13**	**44.00**
	UO	**1.20**	**0.88**	**4.62**	**0.58**	**64.00**
1	C_5	0.91	0 63	1.64	0.34	52.20
2	C_5	0.99	0.33	2.64	0.03	61.10
3	C_5	1.32	0.33	2.37	0.14	44.40
4	C_5	1.06	0 81	4.05	0.81	321.55
5	C_5	0.99	0.10	4.58	0.01	475.57
6	C_5	1.20	1.09	3.59	1.45	82.07
7	C_5	0.81	0 47	2.25	0.07	692.50
8	C_5	1.88	1.18	1.85	0.80	43.93
9	C_5	0.91	0.29	2.20	0.18	65.32
10	C_5	0.69	0.45	3.33	0.10	80.30
11	C_5	0.66	0.74	2.60	0.64	60.30
12	C_5	0.76	0.19	2.38	0.02	10.50
13	C_5	1.45	0 37	2.18	0.51	23.00
14	C_5	0.79	0.78	2.56	0.22	60.30
15	C_5	1.52	0.42	3.05	0.23	50.00
16	C_5	0.79	tonometer fell			
17	C_5	1.62	0.79	2.22	0.43	36.00
18	C_5	0.89	0.26	2.12	0 02	15.00
19	C_5	0 69	0.31	2.15	0.01	21.50
20	C_5	0.66	0.12	4.20	0.01	12.90
21	C_5	0.86	0.79	5 66	0.43	31.00
22	C_5	0.56	0.76	1.99	0.75	32 60
23	C_5	1.42	0.31	2.79	0.16	100.40
24	C_5	1.75	1.38	5.95	1.37	111.70
	median	**0.91**	**0.45**	**2.56**	**0.22**	**52.20**
	LO	**0.78**	**0.31**	**2.19**	**0.05**	**31.80**
	UO	**1.35**	**0.79**	**3.46**	**0.58**	**81.18**
1	C_6	2.11	1.09	2.28	2.27	52.40
2	C_6	0.99	0.26	3.07	1.01	125.40
3	C_6	0.89	0.62	3.27	0.57	67.90
4	C_6	1.01	1.05	2.77	0.93	66.83
5	C_6	0.96	0.35	3.74	0.21	74.10
6	C_6	1.09	0.48	4.26	0.26	33.00
7	C_6	1.55	1.43	5.32	1.63	118.80
8	C_6	0.46	0.60	2.38	0.73	101.20
9	C_6	0.54	0.24	2.16	0.32	129.90
10	C_6	0.94	0.48	3.28	0.43	47.83
11	C_6	0.92	0.78	2.35	0 48	53.9
12	C_6	1.12	0.45	4.14	0.71	79.2
13	C_6	0.36	0.16	3.55	0.06	31.50
14	C_6	0.92	0.84	2.58	0.05	59.2
15	C_6	0.99	0.41	2.43	0.22	74.3
16	C_6	1.32	0.57	9.03	0.59	68.20
17	C_6	1.06	1.05	3.23	0.37	38.00
18	C_6	0.26	0.24	2.73	0.29	108.90
19	C_6	1.22	0.03	4.63	0.30	3.60
20	C_6	0.49	0.54	4 07	0.49	71.70
	median	**0.97**	**0.51**	**3.25**	**0.43**	**68.05**
	LO	**0.80**	**0.28**	**2.74**	**0.30**	**45.38**
	UO	**1.12**	**0.94**	**3.99**	**0.83**	**103.13**

Median and inter-quartile ranges of tissue tonometry parameters in limbs of normal controls and in patients with lower limb chronic venous disease.

Limb no	CEAP Clinical Classification	Phase I (C_2) (spring constant)	Phase II (C_1) (spring constant)	Phase II (k) Dashpot constant	Phase III (C_1) (spring constant)	Phase III (k) Dashpot constant
1	C_0	1.34	10.09	25.62	16.67	762.04
2	C_0	1.07	9.61	28.45	18.57	1008.34
3	C_0	1.26	18.88	56.56	11.90	3878.49
4	C_0	1.28	13.91	26.52	10.66	612.15
5	C_0	0.97	12.18	32.27	31.60	16953.96
6	C_0	0.89	11.75	29.91	44 24	3815.09
7	C_0	1.32	19.60	41.23	11.96	1213 65
8	C_0	1.28	10.77	24.78	51.99	3344.54
9	C_0	1.88	13.15	39.85	32.88	1986.08
10	C_0	1.28	8 89	37.24	5.14	814.35
11	C_0	1.44	9.96	36.07	82.21	12750.06
12	C_0	1.06	12.18	33.73	27.40	19222.37
13	C_0	1.42	8 02	17.16	32.88	28337.85
14	C_0	1.36	8.43	20.40	54.80	3441.67
15	C_0	1.19	164 41	945.36	27.40	29169.22
16	C_0	1.35	19.34	39.07	328.82	24957.56
17	C_0	2.08	2.63	4.08	17.31	1716.80
18	C_0	1.02	12.18	26.67	27.40	1589.30
19	C_0	1.38	18.27	43.66	41.10	1676.99
20	C_0	1.25	8 89	15.73	548.04	61599.26
	median	**1.28**	**11.96**	**31.09**	**29.50**	**3393.10**
	LO	**1.16**	**9.43**	**25.41**	**17.15**	**1495.39**
	UO	**1.37**	**15.00**	**39.27**	**46.18**	**17521.06**
1	C_1	0.92	5.77	13.15	9.96	591.88
2	C_1	1.17	7.47	16.67	328.82	163062.66
3	C_1	1.25	10.58	30.16	27.81	2306.15
4	C_1	1.31	9.72	19 99	14.83	1006.17
5	C_1	1.42	10.00	20.61	19.85	4200.42
6	C_1	0.93	12.46	37.24	10.68	734.51
7	C_1	0.92	5.26	14.23	18.15	2932.21
8	C_1	1.42	27.42	49.82	420.49	39253 04
9	C_1	0.75	19.26	60.22	24.47	22112.16
10	C_1	0.79	26.11	132.88	48.95	78446.61
11	C_1	0.73	8 22	13.48	32.88	2854.17
12	C_1	1.10	11.74	25.01	19.34	3765.59
13	C_1	0.83	11.34	36.06	65.76	4452.25
14	C_1	0.93	7.00	14.69	3.91	176.94
15	C_1	1.05	9.96	23.91	328.82	48994.43
16	C_1	1.12	6.85	10 89	12.18	3165.21
17	C_1	1.64	8 65	21.98	27.40	1123.47
18	C_1	1.35	32.88	101.28	164.41	24612.30
19	C_1	1.60	32.88	117.06	164.41	7332.72
20	C_1	1.07	9.13	21.37	15.66	703 05
21	C_1	2.37	25.29	67.03	164.41	19219.63
22	C_1	1.75	17.31	84.80	9.67	4707.95
23	C_1	1.66	23.49	50.73	29.89	1542.47
24	C_1	2.94	16.78	51.67	16.12	12862.73
25	C_1	1.13	15.66	26.93	36.54	113772.29
26	C_1	0.99	10.28	45.93	328.82	39294.19
	median	**1.12**	**10.96**	**28.54**	**27.60**	**4326.33**
	LO	**0.93**	**8.77**	**20.15**	**15.77**	**1733.39**
	UO	**1.42**	**18.77**	**51.44**	**139.75**	**23987.27**
1	C_2	1.08	2.67	4.81	13.70	2462.05
2	C_2	1.29	4.77	11.53	3 69	229.07
3	C_2	0.87	4.11	8.80	4.38	257.80
4	C_2	1.17	9.77	29.92	12.79	1046.58
5	C_2	1.10	8.79	25 67	8.52	921.06
6	C_2	1.11	9.49	35.20	8.61	565.14
7	C_2	0.94	13.00	44.71	11.17	858.23
8	C_2	1.13	6.33	33.32	7.51	587.87
9	C_2	1.33	7.57	14.91	15.41	2037.82
10	C_2	1.42	7.83	16.13	164.41	22326.99
11	C_2	1.38	27.40	82.48	54.80	4373.33
12	C_2	1.72	8.89	31.55	54.80	608.32
13	C_2	0.93	6.20	16.69	164 41	21587.14
14	C_2	2.27	14.30	30.17	32.88	2107.75
15	C_2	2.16	7.65	20.26	164 41	18923.69
16	C_2	0.79	5.57	12.15	23.49	1707.52
17	C_2	1.49	20.55	63.09	4.11	87.55
	median	**1.17**	**7.83**	**25.67**	**13.70**	**1046.58**
	LO	**1.08**	**6.20**	**14.91**	**8.52**	**587.87**
	UO	**1.42**	**9.77**	**33.32**	**54.80**	**2462.05**
1	C_3	1.31	12.65	24.03	7.31	517.35

2	C_3	0.91	7.31	24.11	6.20	1086.97
3	C_3	1.26	4.91	19.14	4.84	434.72
4	C_3	1.59	7.26	29.99	8.19	885.76
5	C_3	1.14	5.18	13.78	7.14	639.64
6	C_3	0.96	6.47	19.39	10.11	2426.31
7	C_3	1.40	16.44	104.57	6.58	280.16
8	C_3	1.17	5.98	25.11	6.58	664.22
9	C_3	1.29	5.29	14.19	10.24	727.86
10	C_3	0.93	4.33	20.50	89.47	7950.47
11	C_3	0.95	6.33	18.62	13.06	1065.74
12	C_3	1.03	5.30	9.39	11.34	1133.87
13	C_3	1.13	3.87	7.43	4.06	199.73
14	C_3	2.44	6.09	24.42	5.98	400.56
15	C_3	1.42	6.85	76.04	11.74	506.15
16	C_3	2.12	4.01	30.60	3.65	267.08
17	C_3	3.10	1.98	4.54	1.44	76.25
18	C_3	3.02	5.06	18.06	9.67	527.08
19	C_3	1.05	4.57	11.97	5.77	239.98
20	C_3	1.06	2.83	12.59	1.90	84.77
21	C_3	2.22	14.95	26.60		
22	C_3	2.70	2.55	7.11	1.22	182.68
23	C_3	1.66	8.65	21.81	7.15	386.72
24	C_3	1.09	7.31	33.69	3.26	175.81
25	C_3	0.86	4.98	19.78	3.57	700.53
	median	**1.26**	**5.30**	**19.78**	**6.58**	**511.75**
	LO	**1.05**	**4.57**	**13.78**	**3.96**	**260.30**
	UO	**1.66**	**7.26**	**25.11**	**9.78**	**767.34**
1	C_{4a}	1.33	11.34	35.04	3.65	142.85
2	C_{4a}	1.49	8.22	25.24	6.45	384.27
3	C_{4a}	1.14	3.58	6.77	4.08	292.33
4	C_{4a}	1.31	3.33	13.83	3.79	294.43
5	C_{4a}	1.22	5.39	14.72	5.57	339.97
6	C_{4a}	0.95	4.01	19.77	5.06	281.77
7	C_{4a}	1.49	10.60	36.90	13.20	653.27
8	C_{4a}	1.09	6.20	39.15	4.27	276.30
9	C_{4a}	1.12	4.42	13.30	14.91	2043.60
10	C_{4a}	1.26	4.94	9.80	4.75	345.48
11	C_{4a}	1.15	5.57	47.87	2.88	645.24
12	C_{4a}	1.13	4.91	21.79	4.01	224.56
13	C_{4a}	0.91	3.54	8.98	4.98	250.10
14	C_{4a}	1.69	7.47	34.38	6.45	131.53
15	C_{4a}	1.36	6.85	16.10	20.55	1163.21
16	C_{4a}	1.66	3.54	16.37	4.57	256.21
17	C_{4a}	1.02	8.89	30.04	12.18	761.16
18	C_{4a}	1.47	4.11	28.81	3.50	203.24
19	C_{4a}	1.44	3.69	8.28	14.95	838.50
20	C_{4a}	0.98	3.82	8.87	328.82	7266.96
21	C_{4a}	1.12	7.31	19.00	10.96	928.37
22	C_{4a}	1.49	5.77	22.09	10.61	362.76
23	C_{4a}	1.88	6.85	30.35	6.58	308.43
	median	**1.26**	**5.39**	**19.77**	**5.57**	**339.97**
	LO	**1.12**	**3.92**	**13.56**	**4.18**	**266.25**
	UO	**1.48**	**7.08**	**30.19**	**11.57**	**707.22**
1	C_{4b}	3.83	7.65	30.66	5.98	168.00
2	C_{4b}	4.15	5.30	10.45	29.89	2077.56
3	C_{4b}	4.53	3.46	17.62	2.51	490.72
4	C_{4b}	3.21	4.63	10.56	6.45	929.08
5	C_{4b}	2.69	3.50	17.98	5.39	292.71
6	C_{4b}	2.14	2.84	6.20	5.08	253.71
7	C_{4b}	2.26	3.56	11.84	4.26	180.81
8	C_{4b}	4.98	8.66	16.93	18.12	2786.12
9	C_{4b}	3.69	4.44	16.66	27.40	1603.01
10	C_{4b}	2.93	4.50	23.29	25.29	1383.58
11	C_{4b}	2.86	7.31	27.84	6.45	341.72
12	C_{4b}	1.91	5.77	13.96	4.77	254.00
13	C_{4b}	5.57	16.44	46.04	109.61	4998.09
14	C_{4b}	5.87	5.98	22.06	17.31	536.50
15	C_{4b}	4.50	4.70	10.01	7.83	236.44
16	C_{4b}	7.15	5.67	13.55	15.66	505.76
17	C_{4b}	3.02	3.13	10.12	8.89	344.82
18	C_{4b}	7.65	11.34	52.61	36.54	1698.91
19	C_{4b}	5.87	5.87	14.97	8.89	253.28
20	C_{4b}	2.76	3.13	8.33	3.61	284.02
21	C_{4b}	4.01	8.22	20.39	32.88	595.17
22	C_{4b}	3.57	14.95	41.40	109.61	8132.86
23	C_{4b}	3.82	7.31	27.55	11.74	560.17
24	C_{4b}	2.63	7.47	24.89	14.30	703.39
25	C_{4b}	5.87	20.55	51.99		

26	C_{4h}	2.86	5.57	26.86	6.58	366.31
	median	3.76	5.72	17.80	8.89	505.76
	LO	2.86	4.46	12.27	5.98	284.02
	UO	4.87	7.60	27.38	25.29	1383.58
1	C_5	3.60	5.20	8.53	9.70	506.51
2	C_5	3.32	9.96	26.31	109.61	6697.00
3	C_5	2.49	9.96	23.62	23.49	1042.83
4	C_5	3.11	4.08	16.51	4.08	1311.03
5	C_5	3.32	31.78	145.45	402.56	191442 89
6	C_5	2.74	3.01	10.80	2.27	186.44
7	C_5	4.04	6.93	15.57	45.05	31199.09
8	C_5	1.75	2.80	5.17	4.09	179.54
9	C_5	3.60	11.54	25.38	18.27	1193.32
10	C_5	4.74	7.31	24.32	32.12	2579 07
11	C_5	4.98	4.44	11.55	5.14	309.81
12	C_5	4.33	17.31	41.19	164.41	1726.31
13	C_5	2.27	8.89	19.37	6.45	148.29
14	C_5	4.16	4.22	10.79	14.95	901.27
15	C_5	2.16	7.83	23.88	14.61	730.71
16	C_5	4.16				
17	C_5	2.03	4.16	9.24	7.65	275.29
18	C_5	3.69	12.65	26.81	164.41	2466.16
19	C_5	4.77	10.61	22.81	328.82	7069.67
20	C_5	4.98	27.40	115.09	328.82	4241.80
21	C_5	3.82	4.16	23.56	7.65	237.06
22	C_5	5.87	4.33	8.61	4.38	142.93
23	C_5	2.32	10.61	29.59	20.55	2063.36
24	C_5	1.88	2.38	14.18	2.40	268.10
	median	3.60	7.31	22.81	14.95	972.05
	LO	2.45	4.19	11.18	5.79	260.34
	UO	4.20	10.61	25.84	77.33	2494.39
1	C_6	1.56	3.02	6.88	1.45	75.90
2	C_6	3.32	12.65	38 83	3.26	408.26
3	C_6	3.69	5.28	17.24	5.79	393.14
4	C_6	3.25	3.13	8.65	3.52	235.58
5	C_6	3.44	9.39	35.14	15.66	1160.27
6	C_6	3.02	6.85	29.18	12.65	417.35
7	C_6	2.12	2.30	12.23	2.02	239.66
8	C_6	7.12	5.48	13.04	4.50	455.85
9	C_6	6.04	13.47	29.09	10.44	1355.84
10	C_6	3.52	6.89	22.61	7.65	365.78
11	C_6	3.57	4.22	9.91	6.85	369.24
12	C_6	2.94	7.31	30.25	4 63	366.80
13	C_6	9.13	20.55	72.96	54.80	1726.31
14	C_6	3.57	3.91	10.10	65.76	3893.25
15	C_6	3.32	8 02	19.49	14.95	1110.52
16	C_6	2.49	5.77	52.09	5.57	380.10
17	C_6	3.10	3.13	10.12	8.89	337.71
18	C_6	12.65	13.70	37.40	11.34	1234.78
19	C_6	2.70	109.61	507.48	10.96	39.46
20	C_6	6.71	6 09	24.78	6.71	481.15
	median	3.38	6.47	23.70	7.25	400.70
	LO	3.00	4.14	11.70	4.60	358.76
	UO	4.28	10.21	35.70	11.67	1122.96

Median and inter-quartile ranges of spring and dashpot constants calculated from the Kelvin-Hooke model in limbs of normal controls and in patients with lower limb chronic venous disease.

Appendix VI

Study IV

Pt No	Age	Leg	CEAP Clinical Classification	CEAP LDS severity score	Xo (mm)	Series Hooke element C_2 (spring constant)
1	51	R	C_{4b}	2	1.15	2.86
1	51	L	C_{4b}	1	1.72	1.91
2	78	R	C_6	2	0.59	5.58
2	78	L	C_{4b}	2	0.56	5.88
3	63	R	C_6	2	0.73	4.51
3	63	L	C_{4b}	2	0.46	7.15
4	65	R	C_{4b}	1	1.09	3.02
4	65	L	C_5	2	0.43	7.65
5	70	R	C_{4b}	2	0.86	3.83
5	70	L	C_{4b}	2	1.09	3.02
6	69	R	C_{4b}	2	0.56	5.88
6	69	L	C_{4b}	1	1.19	2.76
7	69	R	C_{4b}	1	1.52	2.16
7	69	L	C_6	2	0.79	4.16
8	43	R	C_6	2	0.89	3.70
8	43	L	C_{4b}	1	1.62	2.03
9	57	R	C_5	2	0.86	3.83
9	57	L	C_5	2	0.56	5.88
10	77	R	C_{4b}	1	1.42	2.32
10	77	L	C_5	1	1.75	1.88
11	63	R	C_{4b}	1	1.53	2.14
11	63	L	C_{4b}	2	1.45	2.27
12	72	R	C_{4b}	2	0.89	3.69
12	72	L	C_{4b}	1	1.12	2.93
13	71	R	C_5	2	0.91	3.60
13	71	L	C_6	2	0.94	3.52
14	75	R	C_6	1	1.55	2.12
14	75	L	C_6	2	0.46	7.12
15	50	R	C_{4b}	2	0 66	4.98
15	50	L	C_5	1	1.32	2.49
16	52	R	C_{4b}	2	0.73	4.53
16	52	L	C_6	2	0.99	3.32

Correlation of tissue tonometry parameters of skin compliance with clinical assessment of the severity of skin changes in patients with bilateral LDS.

Appendix VII

Study V

Limb no	CEAP	Skin (mm)	Subcutaneous layer (mm)
1	C_0	3.3	5.35
2	C_0	2.5	8.3
3	C_0	2.64	5.1
4	C_0	3.2	13.9
5	C_0	3	8.9
6	C_0	2.8	8.3
7	C_0	2.7	4.5
8	C_0	2.5	10.8
9	C_0	2.4	7
10	C_0	2.5	7.5
11	C_0	2.8	7
12	C_0	3.5	5.6
Median	**Median**	**2.75**	**7.25**
UO	**UO**	**3.05**	**8.45**
LO	**LO**	**2.5**	**5.54**
13	C_2	3.1	11.5
14	C_2	3.6	12.8
15	C_2	1.5	15.6
16	C_2	1.4	29.4
17	C_2	3.2	16
18	C_2	3.3	?
19	C_2	3.5	9.8
Median	**Median**	**3.2**	**14.2**
UO	**UO**	**3.4**	**15.9**
LO	**LO**	**2.3**	**11.8**
20	C_{4a}	2.6	11.2
21	C_{4a}	3.2	12
22	C_{4a}	2.3	6.6
23	C_{4a}	1.5	15.6
24	C_{4a}	1.6	17.9
Median	**Median**	**2.3**	**12**
UO	**UO**	**2.6**	**15.6**
LO	**LO**	**1.6**	**11.2**
25	C_{4b}	6.4	?
26	C_{4b}	5	?
27	C_{4b}	1.3	11.4
28	C_{4b}	1	12.3
29	C_{4b}	3.3	8.2
30	C_{4b}	3.6	4.6
31	C_{4b}	1	?
32	C_{4b}	1	10.8
33	C_{4b}	1.3	14.3
34	C_{4b}	1.1	10.5
35	C_{4b}	1.1	14.4
36	C_{4b}	0.8	12.7
37	C_{4b}	1.8	?
38	C_{4b}	4.6	9.1
39	C_{4b}	4	?
40	C_{4b}	1.5	11.6
41	C_{4b}	1.5	10.6
42	C_{4b}	4.4	7
43	C_{4b}	6.6	8.8
44	C_{4b}	6.7	15.1
45	C_{4b}	1.8	13.5
46	C_{4b}	3.5	4.6
47	C_{4b}	0.8	?
48	C_{4b}	0.7	5.8
49	C_{4b}	1	24.6
Median	**Median**	**1.5**	**10.8**
UO	**UO**	**4**	**13.1**
LO	**LO**	**1**	**8.5**

Median and Interquartile ranges of the ultrasound measurements of skin and subcutaneous layer thickness in patients with lower limb chronic venous disease using a 7MHz linear array transducer probe.

		Tissue tonometry PHASE I (0-1s)	Tissue tonometry PHASE II (1-10s)		Tissue tonometry PHASE III (10-300s)	
Limb No	CEAP Clinical Classification	X_O (mm)	$X_I - X_O$ (mm)	-1/Tau (secs $^{-1}$)	$X_I - X_O$ (mm)	-1/Tau (secs $^{-1}$)
1	C_0	1.75	0.25	3.03	0.1	60.4
2	C_0	2.57	0.37	4.19	0.64	158.5
3	C_0	2.28	0.33	3.62	0.04	155.1
4	C_0	3.1	0.27	2.77	0.12	701.5
5	C_0	2.31	0.41	2.14	0.1	861.8
6	C_0	2.41	0.39	2.42	0.06	62.8
7	C_0	2.44	0.17	2.02	0.01	75.9
8	C_0	3.96	0.29	3.18	0.05	67.7
9	C_0	3.53	0.47	2.1	0.84	45.2
10	C_0	3.14	0.33	2.4	0.01	149
11	C_0	3.07	0.36	2.34	0.21	44.9
12	C_0	1.98	0.14	2.16	0.11	51.6
13	C_2	2.38	0.12	3.01	0.06	79.8
14	C_2	3.2	0.62	1.77	0.29	100
15	C_2	1.91	0.37	3.55	0.06	11.1
16	C_2	2.9	0.85	1.92	0.81	49.2
17	C_2	1.35	0.54	4.01	0.55	67
18	C_2	2.31	0.48	11.1	0.28	43.1
19	C_2	3.1	1.16	4.44	1.73	44.6
20	C_{4a}	2.41	0.48	2.35	0.16	56.6
21	C_{4a}	1.98	0.93	4.63	0.72	56.1
22	C_{4a}	3.23	0.37	3.38	0.27	62.5
23	C_{4a}	2.82	0.66	4.39	0.83	56.1
24	C_{4a}	3.76	0.9	2.58	0.66	47.7
25	C_{4b}	1.15	0.45	3.81	0.51	53.0
26	C_{4b}	1.72	0.57	2.42	0.69	53.3
27	C_{4b}	0.59	0.20	2.80	0.03	45.6
28	C_{4b}	0.56	0.55	3.69	0.19	31
29	C_{4b}	0.73	0.70	2.13	0.42	30.2
30	C_{4b}	0.46	0.58	2.39	0.21	32.3
31	C_{4b}	1.09	1.05	3.23	0.37	38.8
32	C_{4b}	0.43	0.29	4.64	0.09	46.5
33	C_{4b}	0.86	0.40	2.95	0.09	27.8
34	C_{4b}	1.09	0.59	4.28	0.54	56.6
35	C_{4b}	0.56	0.56	2.55	0.37	28.5
36	C_{4b}	1.19	1.05	2.66	0.91	78.6
37	C_{4b}	1.12	0.45	4.14	0.71	79.2
38	C_{4b}	0.99	0.41	2.43	0.22	74.3
39	C_{4b}	1.32	0.57	9.03	0.59	68.2
40	C_{4b}	1.06	1.05	3.23	0.37	38
41	C_{4b}	0.26	0.24	2.73	0.29	108.9
42	C_{4b}	1.22	0.03	4.63	0.3	3.6
43	C_{4b}	0.49	0.54	4.07	0.49	71.7
44	C_{4b}	0.66	0.74	2.6	0.64	60.3
45	C_{4b}	0.76	0.19	2.38	0.02	10.5
46	C_{4b}	1.45	0.37	2.18	0.51	23
47	C_{4b}	0.86	0.45	3.77	0.28	47.7
48	C_{4b}	1.25	0.44	3.33	0.23	49.2
49	C_{4b}	0.56	0.16	2.53	error	error

Tissue tonometry analysis of distance and rate constant parameters in patients with lower limb chronic venous disease.

Appendix VIII

Study VI

	Phase I		Phase II				Phase III			
Limb No	X_O mm)	X_O (mm)	X_I-X_O (mm)	X_I-X_O (mm)	-1/Tau (secs $^{-1}$)	-1/Tau (secs $^{-1}$)	XI-XO (mm)	XI-XO (mm)	-1/Tau (secs $^{-1}$)	-1/Tau (secs $^{-1}$)
	pre-	post-	pre-	post-	pre-	post-	pre-	post-	pre-	post-
1	2.81	2.67	0.44	0.47	2.23	5.39	0.01	0.48	495.9	68.37
2	2.09	2.92	0.63	0.62	2.99	2.65	0.64	0.51	66.93	124.53
3	2.23	2.22	1.11	0.52	2.93	1.67	0.82	0.38	73.73	73.90
4	1.81	2.83	1.36	0.46	3.13	3.48	0.86	0.49	53.03	83.73
5	1.55	2.67	1.43	0.65	5.32	2.52	1.63	0.53	118.8	59.00
6	0.46	2.31	0.60	0.45	2.38	2.94	0.73	0.26	101.2	65.20
7	0.96	1.42	0.35	0.47	3.74	2.33	0.21	0.30	74.10	65.90
8	1.02	1.25	0.71	0.44	2.28	3.07	0.51	0.91	144.1	44.40
9	0.45	0.79	0.25	0.40	2.74	2.52	0.18	0.17	114.1	105.9
10	0.99	1.32	0.40	0.41	2.45	3.54	0.12	0.36	74.13	91.07
11	3.80	4.36	0.80	1.14	2.14	15.08	0.75	0.12	58.80	52.70
12	2.11	2.87	1.09	0.32	2.28	3.06	2.26	1.48	52.40	33.70
13	3.04	1.95	1.23	0.35	1.80	4.75	0.24	0.69	179.7	41.70
14	0.86	1.55	0.43	0.38	4.01	2.39	0.55	2.02	28.10	38.60
15	0.79	2.38	0.62	0.47	1.97	8.54	0.11	0.85	69.50	45.80
16	2.54	3.30	0.69	0.39	2.42	4.01	0.89	0.54	62.00	66.80
median	1.68	2.34	0.66	0.46	2.44	3.07	0.60	0.50	73.92	65.55
LO	0.94	1.52	0.44	0.40	2.27	2.52	0.20	0.35	61.20	45.45
UO	2.31	2.84	1.10	0.48	3.03	4.20	0.83	0.73	115.3	76.36

Tissue tonometry parameters of the mechanical properties of the skin in patients with lower limb chronic venous disease before and after compression stocking wear.

	C_2	C_1	k	C_1	k
Limb No	(spring constant)	(spring constant)	Dashpot constant	(spring constant)	Dashpot constant
	Phase I	Phase II	Phase II	Phase III	Phase III
1	1.17	7.48	16.67	411.25	203938.88
2	1.57	5.22	15.61	5.14	344.06
3	1.48	2.96	8.68	4.01	295.82
4	1.82	2.42	7.57	3.83	202.87
5	2.12	2.30	12.24	2.02	239.79
6	7.12	5.48	13.05	4.51	456.09
7	3.43	9.40	35.16	15.67	1160.90
8	3.22	4.63	10.57	6.45	929.59
9	7.31	13.16	36.06	18.28	2085.49
10	3.32	8.23	20.15	27.42	2032.40
11	0.87	4.11	8.80	4.39	257.94
12	1.56	3.02	6.88	1.46	76.28
13	1.08	2.67	4.81	13.71	2463.39
14	3.83	7.65	30.68	5.98	168.09
15	4.15	5.31	10.45	29.91	2078.68
16	1.29	4.77	11.54	3.70	229.19
median	1.97	5.00	11.89	5.56	400.08
LO	1.43	3.00	8.77	3.97	237.14
UO	3.53	7.52	17.54	16.32	2043.97

Median and inter-quartile range values for spring and dashpot constant parameters before compression stocking wear.

	C_2	C_1	k	C_1	k
Limb No	spring constant	spring constant	Dashpot constant	spring constant	Dashpot constant
	Phase I	Phase II	Phase II	Phase III	Phase III
1	1.23	7.00	37.73	6.85	468.62
2	1.13	5.31	14 06	6.45	803.34
3	1.48	6.33	10.57	8.66	639.82
4	1.16	7.15	24 89	6.71	562.19
5	1.23	5 06	12.76	6.21	366 25
6	1.42	7.31	21.49	12.65	825.03
7	2.32	7.00	16.31	10.97	722.70
8	2.62	7.48	22.96	3.62	160.52
9	4.16	8.23	20.73	19.35	2049.48
10	2.49	8.02	28.41	9.14	832.28
11	0.75	2.89	43.52	27.42	1444.86
12	1.15	10.28	31.46	2.22	74.91
13	1 69	9.40	44 65	4.77	198.83
14	2.12	8.66	20 69	1.63	62.87
15	1.38	7.00	59.78	3.87	177.27
16	1.00	8.44	33.83	6.09	406.99
median	1.40	7.23	23.92	6.58	515.40
LO	1.16	6.83	19.60	4.54	193.44
UO	2.17	8.28	34.80	9.60	808.76

Median and inter-quartile range values for spring and dashpot constant parameters after compression stocking wear.

	CIRCUMFERENTIAL LIMB VOLUME MEASUREMENT (ml)	
	Disc model method	
Limb. No	pre-stocking	post-stocking
1	2621	2610.2
2	2547	2431.1
3	2845.5	2421.9
4	2798.1	2369.5
5	2607.7	2116.6
6	2482.8	2138.2
7	2373.4	2069.9
8	2369.5	2213.3
9	2316.5	2034.4
10	2574.8	2257.8
11	2397.1	2132.6
12	2522.1	2314.5
13	2387	2350.3
14	2687.1	2478.5
15	2508	2200.6
16	2257.8	2109.8

Mean value of the circumferential limb volume measurements using a tape measure before and after compression stocking wear.

Appendix IX

Study VII

Method of calculating the Kappa measure of agreement between different examiners using the CEAP method of classification and scoring of lower limb chronic venous disease.

INTER-VARIABILITY OF THE CEAP METHOD OF CLASSIFICATION AND SCORING OF LOWER LIMB VENOUS DISEASE

						CEAP Method of classification				CEAP Method of scoring		
Pt No	Age	Sex	Limb	Clinical	A/S	Etiologic	Anatomic	Pathophysiologic	Clinical	Disability	Anatomic	Examiner
1	25	F	R	C_0	A	E	A	P	0	0	0	A
1	25	F	R	C_0	A	E	A	P	0	0	0	B
1	25	F	R	C_0	A	E	A	P	0	0	0	C
1	25	F	L	C_0	A	E	A	P	0	0	0	A
1	25	F	L	C_0	A	E	A	P	0	0	0	B
1	25	F	L	C_0	A	E	A	P	0	0	0	C
2	32	M	R	C_0	A	E	A	P	0	0	0	A
2	32	M	R	C_0	A	E	A	P	0	0	0	B
2	32	M	R	C_0	A	E	A	P	0	0	0	C
2	32	M	L	C_0	A	E	A	P	0	0	0	A
2	32	M	L	C_0	A	E	A	P	0	0	0	B
2	32	M	L	C_0	A	E	A	P	0	0	0	C
3	55	M	R	C_0	A	E	A	P	0	0	0	A
3	55	M	R	C_0	A	E	A	P	0	0	0	B
3	55	M	R	C_0	A	E	A	P	0	0	0	C
3	55	M	L	C_0	A	E	A	P	0	0	0	A
3	55	M	L	C_0	A	E	A	P	0	0	0	B
3	55	M	L	C_0	A	E	A	P	0	0	0	C
4	42	F	R	C_0	A	E	A	P	0	0	1	A
4	42	F	R	C_1	A	E	A	P	0	0	1	B
4	42	F	R	C_0	A	E	A	P	0	0	1	C
4	42	F	L	C_1	A	E	A	P	0	0	1	A
4	42	F	L	C_1	A	E	A	P	0	0	1	B
4	42	F	L	C_1	A	E	A	P	0	0	1	C
5	53	M	R	C_2	S	E_P	$A_{S,P}$	P_R	1	1	3	A
5	53	M	R	C_2	S	E_P	$A_{S,P}$	P_R	1	1	3	B
5	53	M	R	C_2	S	E_P	$A_{S,P}$	P_R	1	1	3	C
5	53	M	L	C_2	S	E_P	$A_{S,P}$	P_R	1	1	2	A
5	53	M	L	C_2	S	E_P	$A_{S,P}$	P_R	1	1	2	B
5	53	M	L	C_2	S	E_P	$A_{S,P}$	P_R	1	1	2	C
6	72	F	L	C_2	S	E_P	A_S	P_R	1	1	3	A
6	72	F	L	C_2	S	E_P	A_S	P_R	1	1	3	B
6	72	F	L	C_2	S	E_P	A_S	P_R	1	1	3	C
6	72	F	R	C_3	S	E_P	A_S	P_R	3	1	3	A
6	72	F	R	C_3	S	E_P	A_S	P_R	3	1	3	B
6	72	F	R	C_3	S	E_P	A_S	P_R	3	1	3	C
7	56	M	R	C_3	A	E_P	A_S	P_R	1	0	3	A
7	56	M	R	C_3	A	E_P	A_S	P_R	1	0	3	B
7	56	M	R	C_2	A	E_P	A_S	P_R	0	0	3	C
7	56	M	L	C_3	S	E_P	A_S	P_R	2	1	4	A
7	56	M	L	C_3	S	E_P	A_S	P_R	2	1	4	B
7	56	M	L	C_2	S	E_P	A_S	P_R	1	1	4	C
8	47	F	R	C_3	S	E_P	A_S	P_R	2	1	2	A
8	47	F	R	C_3	S	E_P	A_S	P_R	2	1	2	B
8	47	F	R	C_2	S	E_P	A_S	P_R	1	1	2	C
8	47	F	L	C_3	S	E_P	A_S	P_R	2	1	2	A
8	47	F	L	C_3	S	E_P	A_S	P_R	2	1	2	B
8	47	F	L	C_2	S	E_P	A_S	P_R	1	1	2	C
9	62	M	R	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	6	A
9	62	M	R	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	6	B
9	62	M	R	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	6	C
9	62	M	L	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	7	A
9	62	M	L	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	7	B
9	62	M	L	C_4	S	E_S	$A_{S,P,D}$	P_R	3	2	7	C
10	67	F	R	C_4	S	E_S	$A_{S,P,D}$	P_R	4	2	4	A
10	67	F	R	C_4	S	E_S	$A_{S,P,D}$	P_R	4	2	4	B
10	67	F	R	C_4	S	E_S	$A_{S,P,D}$	P_R	4	2	4	C
10	67	F	L	C_4	S	E_P	A_S	P_R	4	2	3	A
10	67	F	L	C_4	S	E_P	A_S	P_R	4	2	3	B
10	67	F	L	C_4	S	E_P	A_S	P_R	4	2	3	C
11	72	F	R	C_4	S	E_P	A_S	P_R	7	2	2	A
11	72	F	R	C_4	S	E_P	A_S	P_R	6	2	2	B
11	72	F	R	C_4	S	E_P	A_S	P_R	6	2	2	C
11	72	F	L	C_4	S	E_P	A_S	P_R	7	2	2	A

11	72	F	L	C_4	S	E_P	As	P_R	7	2	2	B
11	72	F	L	C_4	S	E_P	As	P_R	7	2	2	C
12	75	M	R	C_6	S	Es	$A_{S,D}$	P_R	12	2	5	A
12	75	M	R	C_6	S	Es	$A_{S,D}$	P_R	12	2	5	B
12	75	M	R	C_6	S	Es	$A_{S,D}$	P_R	10	2	5	C
12	75	M	L	C_6	S	E_P	As	P_R	11	2	2	A
12	75	M	L	C_6	S	E_P	As	P_R	11	2	2	B
12	75	M	L	C_6	S	E_P	As	P_R	11	2	2	C
13	63	F	R	C_4	S	E_P	As	P_R	7	2	2	A
13	63	F	R	C_4	S	E_P	As	P_R	7	2	2	B
13	63	F	R	C_4	S	E_P	As	P_R	7	2	2	C
13	63	F	L	C_4	S	E_P	As	P_R	7	2	2	A
13	63	F	L	C_4	S	E_P	As	P_R	7	2	2	B
13	63	F	L	C_4	S	E_P	As	P_R	7	2	2	C
14	71	F	L	C_5	S	E_P	As	P_R	8	2	2	A
14	71	F	L	C_5	S	E_P	As	P_R	8	2	2	B
14	71	F	L	C_5	S	E_P	As	P_R	8	2	2	C
14	71	F	R	C_5	S	E_P	As	P_R	10	2	2	A
14	71	F	R	C_5	S	E_P	As	P_R	10	2	2	B
14	71	F	R	C_4	S	E_P	As	P_R	8	2	2	C

The kappa measure of agreement

This method is used in study VII to assess the agreement between three different observers in assessing the ease of use of the application of the CEAP method of classification and scoring of lower limb chronic venous disease.

The method for the *kappa* measure of agreement is as follows. The observed measure of agreement, I_o, and the corresponding expected measure, I_e, are defined as follows:

n = total no of observations	Examiner A	
Examiner B/C	positive	negative
positive	a	b
negative	c	d

$$I_o = \frac{a+d}{n},$$

$$I_e = \frac{(a+c)(a+b)+(b+d)(c+d)}{n^2},$$

Kappa is defined as the difference between observed and expected agreement as a fraction of the maximum difference:

$$k = \frac{Io - Ie}{1 - I_e}$$

The maximum value of *kappa* is 1, which represents perfect agreement, and *kappa* will take the value zero if there is only chance agreement.

Fleiss et al, recommended that *kappa* values exceeding 0.75 represents excellent agreement, values between 0.40 and 0.75 represents fair to good agreement and values less than 0.40 represents poor agreement.

Calculation of the Kappa measure of agreement

Clinical classification

	Examiner A	
Examiner B	positive	negative
positive	22	1
negative	0	5

$I_o = \frac{27}{28} = 0.96$,

$I_e = \frac{(23)(22) + (6)(5)}{784} = 0.68$,

$k = \frac{0.96 - 0.68}{1 - 0.68} = \frac{0.28}{0.32} = 0.875$

	Examiner A	
Examiner C	positive	negative
positive	10	0
negative	6	12

$I_o = \frac{22}{28} = 0.79$,

$I_e = \frac{(10)(16) + (12)(18)}{784} = 0.48$,

$k = \frac{0.79 - 0.48}{1 - 0.48} = \frac{0.31}{0.52} = 0.60$

Etiological classification

	Examiner A	
Examiner B	positive	negative
positive	20	0
negative	0	8

$I_o = \frac{28}{28} = 1.00$,

$I_e = \frac{(20)(20) + (8)(8)}{784} = 0.59$,

$k = \frac{1.00 - 0.59}{1.00 - 0.59} = \frac{0.41}{0.41} = 1.00$

	Examiner A	
Examiner C	positive	negative
positive	20	0
negative	0	8

$I_o = \frac{28}{28} = 1.00$,

$I_e = \frac{(20)(20) + (8)(8)}{784} = 0.59$,

$k = \frac{1.00 - 0.59}{1.00 - 0.59} = \frac{0.41}{0.41} = 1.00$

A/S classification

	Examiner A	
Examiner B	positive	negative
positive	19	0
negative	0	9

$I_o = \frac{28}{28} = 1.00$,

$I_e = \frac{(19)(19) + (9)(9)}{784} = 0.56$,

$k = \frac{1.00 - 0.56}{1.00 - 0.56} = \frac{0.44}{0.44} = 1.00$

	Examiner A	
Examiner C	positive	negative
positive	19	0
negative	0	9

$I_o = \frac{28}{28} = 1.00$,

$I_e = \frac{(19)(19) + (9)(9)}{784} = 0.56$,

$k = \frac{1.00 - 0.56}{1.00 - 0.56} = \frac{0.44}{0.44} = 1.00$

Anatomical classification

	Examiner A	
Examiner B	positive	negative
positive	20	0
negative	0	8

$I_o = \frac{28}{28} = 1.00$,

$I_e = \frac{(20)(20) + (8)(8)}{784} = 0.59$,

$k = \frac{1.00 - 0.59}{1.00 - 0.59} = \frac{0.41}{0.41} = 1.00$

	Examiner A	
Examiner C	positive	negative
positive	20	0
negative	0	8

$I_o = \frac{28}{28} = 1.00$,

$I_e = \frac{(20)(20) + (8)(8)}{784} = 0.59$,

$k = \frac{1.00 - 0.59}{1.00 - 0.59} = \frac{0.41}{0.41} = 1.00$

Pathophysiological classification

	Examiner A	
Examiner B	positive	negative
positive	20	0
negative	0	8

$I_o = \frac{28}{28} = 1.00$,

$I_e = \frac{(20)(20) + (8)(8)}{784} = 0.59$,

$k = \frac{1.00 - 0.59}{1.00 - 0.59} = \frac{0.41}{0.41} = 1.00$

	Examiner A	
Examiner C	positive	negative
positive	20	0
negative	0	8

$I_o = \frac{28}{28} = 1.00$,

$I_e = \frac{(20)(20) + (8)(8)}{784} = 0.59$,

$k = \frac{1.00 - 0.59}{1.00 - 0.59} = \frac{0.41}{0.41} = 1.00$

Clinical Scoring

	Examiner A	
Examiner B	positive	negative
positive	19	0
negative	1	8

$I_o = \frac{27}{28} = 0.96$,

$I_e = \frac{(19)(20) + (8)(9)}{784} = 0.58$,

$k = \frac{0.96 - 0.58}{1.00 - 0.58} = \frac{0.38}{0.42} = 0.90$

	Examiner A	
Examiner C	positive	negative
positive	13	0
negative	7	8

$I_o = \frac{21}{28} = 0.75$,

$I_e = \frac{(13)(20) + (8)(15)}{784} = 0.48$,

$k = \frac{0.75 - 0.48}{1.00 - 0.48} = \frac{0.27}{0.52} = 0.52$

Disability Scoring

	Examiner A	
Examiner B	positive	negative
positive	20	0
negative	0	8

	Examiner A	
Examiner C	positive	negative
positive	20	0
negative	0	8

$I_o = \frac{28}{28} = 1.00,$

$I_e = \frac{(20)(20) + (8)(8)}{784} = 0.59,$

$k = \frac{1.00 - 0.59}{1.00 - 0.59} = \frac{0.41}{0.41} = 1.00$

$I_o = \frac{28}{28} = 1.00,$

$I_e = \frac{(20)(20) + (8)(8)}{784} = 0.59,$

$k = \frac{1.00 - 0.59}{1.00 - 0.59} = \frac{0.41}{0.41} = 1.00$

Anatomical Scoring

	Examiner A	
Examiner B	positive	negative
positive	22	0
negative	0	6

	Examiner A	
Examiner C	positive	negative
positive	22	0
negative	0	6

$I_o = \frac{28}{28} = 1.00,$

$I_e = \frac{(26)(26) + (6)(6)}{784} = 0.66,$

$k = \frac{1.00 - 0.66}{1.0 - 0.66} = \frac{0.34}{0.34} = 1.00$

$I_o = \frac{28}{28} = 1.00,$

$I_e = \frac{(26)(26) + (6)(6)}{784} = 0.66,$

$k = \frac{1.00 - 0.66}{1.00 - 0.66} = \frac{0.34}{0.34} = 1.00$

APPENDIX X

Study VIII

Photoplethysmography: Refilling time (secs) with no cuffs (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	28.0	C_1	28.0	C_2	30.0	C_3	22.0	C_{4a}	12.6	C_{4b}	3.5	C_5	11.0	C_6	11.6
C_0	28.0	C_1	19.9	C_2	20.2	C_3	20.0	C_{4a}	11.1	C_{4b}	6.0	C_5	7.5	C_6	8.0
C_0	30.0	C_1	14.0	C_2	12.0	C_3	18.5	C_{4a}	6.2	C_{4b}	4.8	C_5	8.5	C_6	8.0
C_0	31.0	C_1	12.6	C_2	8.5	C_3	20.0	C_{4a}	22.2	C_{4b}	3.0	C_5	7.5	C_6	8.2
C_0	22.0	C_1	27.0	C_2	16.0	C_3	9.8	C_{4a}	10.3	C_{4b}	9.0	C_5	6.5	C_6	5.8
C_0	31.0	C_1	16.5	C_2	22.2	C_3	12.6	C_{4a}	4.0	C_{4b}	10.0	C_5	6.0	C_6	5.0
C_0	13.0	C_1	28.0	C_2	8.0	C_3	13.8	C_{4a}	9.0	C_{4b}	11.0	C_5	9.0	C_6	5.0
C_0	22.2	C_1	27.0	C_2	14.0	C_3	8.4	C_{4a}	10.0	C_{4b}	2.0	C_5	4.8	C_6	7.0
		C_1	23.2	C_2	15.0	C_3	5.8	C_{4a}	6.0	C_{4b}	7.2	C_5	5.0	C_6	6.0
		C_1	12.0	C_2	13.9	C_3	25.5	C_{4a}	7.0	C_{4b}	13.2	C_5	5.4	C_6	6.0
						C_3	18.0	C_{4a}	10.6						
								C_{4a}	11.5						
								C_{4a}	13.2						
Median	28.0	Median	21.6	Median	14.5	Median	18.0	Median	10.3	Median	6.6	Median	7.0	Median	6.5
LO	22.2	LO	14.6	LO	12.5	LO	11.2	LO	7.0	LO	3.8	LO	5.6	LO	5.9
UO	30.3	UO	27.0	UO	19.2	UO	20.0	UO	11.5	UO	9.8	UO	8.3	UO	8.0

Resting Ankle Brachial Pressure Indices (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	1.1	C_1	1.1	C_2	1.1	C_3	1.0	C_{4a}	1.0	C_{4b}	1.0	C_5	1.0	C_6	1.1
C_0	1.1	C_1	1.0	C_2	1.0	C_3	1.0	C_{4a}	0.9	C_{4b}	1.0	C_5	1.0	C_6	1.0
C_0	1.1	C_1	1.1	C_2	1.1	C_3	1.1	C_{4a}	1.0	C_{4b}	1.0	C_5	1.0	C_6	1.0
C_0	1.1	C_1	1.1	C_2	1.1	C_3	1.0	C_{4a}	1.0	C_{4b}	1.0	C_5	1.0	C_6	1.0
C_0	1.2	C_1	1.1	C_2	1.0	C_3	1.0	C_{4a}	1.0	C_{4b}	1.0	C_5	1.0	C_6	1.0
C_0	1.1	C_1	1.0	C_2	1.1	C_3	1.1	C_{4a}	1.0	C_{4b}	1.0	C_5	1.1	C_6	1.1
C_0	1.0	C_1	1.1	C_2	1.0	C_3	1.0	C_{4a}	1.1	C_{4b}	1.1	C_5	1.1	C_6	1.0
C_0	1.0	C_1	1.1	C_2	1.1	C_3	1.0	C_{4a}	1.0	C_{4b}	1.0	C_5	1.1	C_6	1.0
		C_1	1.0	C_2	1.0	C_3	1.0	C_{4a}	1.0	C_{4b}	1.0	C_5	1.0	C_6	1.0
		C_1	1.0	C_2	1.0	C_3	1.0	C_{4a}	1.1	C_{4b}	1.0	C_5	1.1	C_6	1.0
						C_3	1.0	C_{4a}	1.0						
								C_{4a}	1.0						
								C_{4a}	1.0						
Median	1.1	Median	1.1	Median	1.0	Median	1.0	Median	1.0	Median	1.0	Median	1.0	Median	1.0
LO	1.0	LO	1.0	LO	1.0	LO	1.0	LO	1.0	LO	1.0	LO	1.0	LO	1.0
UO	1.1	UO	1.1	UO	1.1	UO	1.0	UO	1.0	UO	1.0	UO	1.1	UO	1.0

APG-Derived Outflow Fraction (%) with Superficial Occlusion (Data with Median values / Lower-Quartile and Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	39.3	C_1	50.7	C_2	48.1	C_3	58.1	C_{4a}	56.4	C_{4b}	69.3	C_5	34.2	C_6	50.0
C_0	41.5	C_1	48.8	C_2	39.4	C_3	34.9	C_{4a}	51.9	C_{4b}	56.4	C_5	29.7	C_6	45.8
C_0	48.6	C_1	44.8	C_2	34.0	C_3	38.9	C_{4a}	75.3	C_{4b}	53.3	C_5	27.0	C_6	52.2
C_0	45.9	C_1	34.2	C_2	48.8	C_3	36.0	C_{4a}	63.7	C_{4b}	47.6	C_5	20.1	C_6	51.0
C_0	56.5	C_1	41.4	C_2	50.5	C_3	41.9	C_{4a}	41.9	C_{4b}	39.8	C_5	39.7	C_6	55.3
C_0	49.1	C_1	34.2	C_2	58.7	C_3	44.5	C_{4a}	49.6	C_{4b}	39.3	C_5	67.6	C_6	54.2
C_0	35.3	C_1	43.2	C_2	52.3	C_3	44.2	C_{4a}	44.4	C_{4b}	62.7	C_5	46.6	C_6	74.4
C_0	42.4	C_1	40.2	C_2	64.7	C_3	55.7	C_{4a}	47.9	C_{4b}	20.2	C_5	63.0	C_6	41.7
		C_1	41.6	C_2	40.0	C_3	21.8	C_{4a}	45.8	C_{4b}	78.0	C_5	77.4	C_6	42.7
		C_1	54.2	C_2	46.8	C_3	57.4	C_{4a}	52.5	C_{4b}	89.1	C_5	75.0	C_6	46.2
						C_3	74.4	C_{4a}	41.7						
								C_{4a}	56.0						
								C_{4a}	42.7						
Median	44.1	Median	42.4	Median	48.4	Median	44.2	Median	49.6	Median	54.9	Median	43.1	Median	50.5
LO	40.9	LO	40.5	LO	41.7	LO	37.4	LO	44.4	LO	41.8	LO	30.8	LO	45.9
UO	48.7	UO	47.8	UO	51.8	UO	56.6	UO	56.0	UO	67.7	UO	66.5	UO	53.7

APG-Derived Venous Volume (ml) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	123.3	C_1	122.8	C_2	90.8	C_3	143.1	C_{4a}	74.8	C_{4b}	175.5	C_5	144.8	C_6	94.3
C_0	124.5	C_1		C_2	222.1	C_3	132.0	C_{4a}	158.1	C_{4b}	133.3	C_5	278.7	C_6	85.2
C_0	158.2	C_1	169.1	C_2	112.6	C_3	144.4	C_{4a}	158.8	C_{4b}	153.3	C_5	96.5	C_6	127.2
C_0	128.8	C_1	88.1	C_2	202.2	C_3	114.7	C_{4a}	206.3	C_{4b}	170.4	C_5	267.0	C_6	108.4
C_0	61.3	C_1	93.9	C_2	176.0	C_3	142.2	C_{4a}	155.6	C_{4b}	158.2	C_5	143.1	C_6	136.3
C_0	39.1	C_1	94.0	C_2	157.1	C_3	179.5	C_{4a}	206.7	C_{4b}	165.4	C_5	205.9	C_6	245.7
C_0	128.4	C_1	95.3	C_2	210.0	C_3	227.2	C_{4a}	105.4	C_{4b}	70.1	C_5	173.4	C_6	93.6
C_0	55.7	C_1	109.7	C_2	83.0	C_3	155.4	C_{4a}	108.4	C_{4b}	65.9	C_5	97.9	C_6	105.1
		C_1	49.1	C_2	66.4	C_3	86.2	C_{4a}	212.6	C_{4b}	111.2	C_5	161.3	C_6	177.6
		C_1	145.6	C_2	126.3	C_3	234.3	C_{4a}	216.3	C_{4b}	98.1	C_5	111.2	C_6	239.7
						C_3	95.4	C_{4a}	123.3						
								C_{4a}	127.2						
								C_{4a}	165.4						
Median	123.9	Median	95.3	Median	141.7	Median	143.1	Median	158.1	Median	143.3	Median	153.1	Median	117.8
LO	59.9	LO	93.9	LO	96.3	LO	123.4	LO	123.3	LO	101.4	LO	119.2	LO	97.0
UO	128.5	UO	122.8	UO	195.7	UO	167.5	UO	206.3	UO	163.6	UO	197.8	UO	167.3

APG-Derived Venous Filling Index (ml/sec) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.5	C_1	4.6	C_2	3.7	C_3	4.6	C_{4a}	2.5	C_{4b}	10.1	C_5	4.9	C_6	5.4
C_0	1.3	C_1	0.9	C_2	2.1	C_3	5.3	C_{4a}	5.9	C_{4b}	3.4	C_5	13.6	C_6	3.0
C_0	0.9	C_1	1.7	C_2	2.1	C_3	5.2	C_{4a}	5.8	C_{4b}	4.6	C_5	3.6	C_6	4.4
C_0	0.8	C_1	0.7	C_2	4.2	C_3	6.9	C_{4a}	8.2	C_{4b}	4.2	C_5	8.7	C_6	2.1
C_0	0.7	C_1	1.0	C_2	4.4	C_3	0.9	C_{4a}	7.7	C_{4b}	11.6	C_5	4.2	C_6	3.6
C_0	1.3	C_1	0.9	C_2	1.8	C_3	6.6	C_{4a}	5.7	C_{4b}	8.3	C_5	8.7	C_6	7.4
C_0	0.7	C_1	0.9	C_2	8.7	C_3	11.0	C_{4a}	1.9	C_{4b}	1.2	C_5	6.8	C_6	2.0
C_0	0.3	C_1	1.0	C_2	2.5	C_3	2.0	C_{4a}	9.0	C_{4b}	2.8	C_5	2.7	C_6	6.7
		C_1	0.9	C_2	1.0	C_3	3.4	C_{4a}	6.4	C_{4b}	4.0	C_5	4.5	C_6	8.4
		C_1	1.4	C_2	2.5	C_3	7.3	C_{4a}	8.5	C_{4b}	7.5	C_5	6.0	C_6	5.8
						C_3	3.1	C_{4a}	3.7						
								C_{4a}	5.0						
								C_{4a}	3.3						
Median	0.7	Median	0.9	Median	2.5	Median	5.2	Median	5.8	Median	4.4	Median	5.5	Median	4.9
LO	0.6	LO	0.9	LO	2.1	LO	3.3	LO	3.7	LO	3.5	LO	4.2	LO	3.1
UO	1.0	UO	1.3	UO	4.1	UO	6.7	UO	7.7	UO	8.1	UO	8.2	UO	6.5

APG-Derived Elected Volume (ml) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	64.5	C_1	70.5	C_2	61.5	C_3	70.6	C_{4a}	40.3	C_{4b}	115.3	C_5	68.2	C_6	41.5
C_0	86.8	C_1		C_2	120.0	C_3	88.7	C_{4a}	65.3	C_{4b}	56.9	C_5	72.3	C_6	52.2
C_0	109.5	C_1	97.2	C_2	54.4	C_3	62.6	C_{4a}	118.6	C_{4b}	97.1	C_5	52.2	C_6	60.0
C_0	88.1	C_1	63.3	C_2	135.5	C_3	55.9	C_{4a}	95.5	C_{4b}	83.8	C_5	88.7	C_6	33.1
C_0	35.1	C_1	63.5	C_2	137.5	C_3	95.9	C_{4a}	74.5	C_{4b}	42.7	C_5	69.0	C_6	83.6
C_0	22.7	C_1	51.7	C_2	96.6	C_3	80.6	C_{4a}	69.9	C_{4b}	69.1	C_5	98.0	C_6	101.0
C_0	108.8	C_1	65.1	C_2	121.8	C_3	121.2	C_{4a}	56.4	C_{4b}	28.0	C_5	83.7	C_6	21.1
C_0	50.0	C_1	54.4	C_2	64.9	C_3	103.6	C_{4a}	66.4	C_{4b}	53.7	C_5	39.2	C_6	73.3
		C_1	37.3	C_2	43.6	C_3	21.1	C_{4a}	81.1	C_{4b}	78.6	C_5	119.8	C_6	99.0
		C_1	109.7	C_2	73.7	C_3	126.4	C_{4a}	88.8	C_{4b}	71.0	C_5	33.7	C_6	120.4
						C_3	86.1	C_{4a}	53.3						
								C_{4a}	29.1						
								C_{4a}	67.3						
Median	75.7	Median	63.5	Median	85.2	Median	86.1	Median	67.3	Median	70.1	Median	70.7	Median	66.6
LO	46.3	LO	54.4	LO	62.3	LO	66.6	LO	56.4	LO	54.5	LO	56.2	LO	44.2
UO	93.3	UO	70.5	UO	121.4	UO	99.7	UO	81.1	UO	82.5	UO	87.4	UO	95.2

APG-Derived Election Fraction (%) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	52.3	C_1	57.4	C_2	67.7	C_3	49.3	C_{4a}	53.9	C_{4b}	65.7	C_5	47.1	C_6	44.0
C_0	69.7	C_1	65.5	C_2	54.0	C_3	67.1	C_{4a}	41.3	C_{4b}	42.6	C_5	26.0	C_6	61.2
C_0	69.2	C_1	57.5	C_2	48.3	C_3	43.4	C_{4a}	74.7	C_{4b}	63.3	C_5	54.1	C_6	47.1
C_0	68.4	C_1	71.9	C_2	67.0	C_3	48.7	C_{4a}	46.3	C_{4b}	49.2	C_5	33.2	C_6	30.5
C_0	57.4	C_1	67.6	C_2	78.1	C_3	67.4	C_{4a}	47.9	C_{4b}	27.0	C_5	48.2	C_6	61.3
C_0	58.1	C_1	55.0	C_2	61.5	C_3	44.9	C_{4a}	33.8	C_{4b}	41.8	C_5	47.6	C_6	41.1
C_0	84.7	C_1	68.3	C_2	58.0	C_3	53.3	C_{4a}	53.4	C_{4b}	40.0	C_5	48.2	C_6	22.5
C_0	89.8	C_1	49.6	C_2	78.2	C_3	66.7	C_{4a}	61.2	C_{4b}	81.5	C_5	40.0	C_6	69.7
		C_1	75.3	C_2	65.8	C_3	24.5	C_{4a}	38.1	C_{4b}	70.6	C_5	74.3	C_6	55.8
		C_1	75.3	C_2	58.3	C_3	54.0	C_{4a}	41.0	C_{4b}	72.4	C_5	30.3	C_6	50.2
						C_3	90.3	C_{4a}	43.2						
								C_{4a}	22.9						
								C_{4a}	40.7						
Median	68.8	Median	66.5	Median	63.6	Median	53.3	Median	43.2	Median	56.2	Median	47.3	Median	48.7
LO	57.9	LO	57.4	LO	58.1	LO	46.8	LO	40.7	LO	42.0	LO	34.9	LO	41.8
UO	73.5	UO	71.9	UO	67.5	UO	66.9	UO	53.4	UO	69.4	UO	48.2	UO	59.9

APG-Derived Residual Volume Fraction (%) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	28.8	C_1	31.0	C_2	15.2	C_3	34.9	C_{4a}	42.7	C_{4b}	39.0	C_5	36.8	C_6	50.0
C_0	26.1	C_1	28.2	C_2	15.2	C_3	18.6	C_{4a}	36.8	C_{4b}	42.6	C_5	43.9	C_6	35.7
C_0	19.2	C_1	26.0	C_2	19.8	C_3	44.8	C_{4a}	14.2	C_{4b}	18.4	C_5	29.4	C_6	30.0
C_0	19.1	C_1	19.8	C_2	12.6	C_3	35.0	C_{4a}	36.6	C_{4b}	31.8	C_5	54.8	C_6	43.8
C_0	25.0	C_1	25.9	C_2	7.1	C_3	15.2	C_{4a}	26.1	C_{4b}	46.0	C_5	16.9	C_6	27.3
C_0	39.5	C_1	25.7	C_2	44.9	C_3	30.5	C_{4a}	30.0	C_{4b}	16.5	C_5	15.9	C_6	23.8
C_0	28.2	C_1	33.7	C_2	26.4	C_3	31.1	C_{4a}	25.9	C_{4b}	36.0	C_5	42.4	C_6	57.8
C_0	27.1	C_1	45.1	C_2	30.8	C_3	5.3	C_{4a}	31.9	C_{4b}	21.0	C_5	24.2	C_6	18.0
		C_1	20.4	C_2	27.4	C_3	64.9	C_{4a}	15.8	C_{4b}	16.5	C_5	31.0	C_6	15.6
		C_1	12.7	C_2	39.6	C_3	36.4	C_{4a}	32.1	C_{4b}	13.3	C_5	36.7	C_6	15.3
						C_3	45.6	C_{4a}	55.3						
								C_{4a}	75.7						
								C_{4a}	35.7						
Median	26.6	Median	25.9	Median	23.1	Median	34.9	Median	32.1	Median	26.4	Median	33.8	Median	28.7
LO	23.6	LO	21.7	LO	15.2	LO	24.6	LO	26.1	LO	17.0	LO	25.5	LO	19.5
UO	28.4	UO	30.3	UO	29.9	UO	40.6	UO	36.8	UO	38.2	UO	41.0	UO	41.7

APG-Derived Arterial Inflow (ml/min) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	41.2	C_1	67.3	C_2	79.8	C_3	121.2	C_{4a}	10.5	C_{4b}	89.7	C_5	85.3	C_6	93.4
C_0	34.2	C_1	48.1	C_2	82.1	C_3	92.1	C_{4a}	86.2	C_{4b}	132.3	C_5	83.3	C_6	48.6
C_0	57.5	C_1	78.8	C_2	112.1	C_3	139.3	C_{4a}	196.0	C_{4b}	151.4	C_5	43.6	C_6	67.8
C_0	47.0	C_1	54.5	C_2	82.1	C_3	58.8	C_{4a}	33.0	C_{4b}	193.9	C_5	103.7	C_6	63.6
C_0	30.8	C_1	76.4	C_2	105.3	C_3	46.4	C_{4a}	59.5	C_{4b}	52.9	C_5	105.4	C_6	130.9
C_0	30.0	C_1	37.8	C_2	44.3	C_3	64.5	C_{4a}	94.4	C_{4b}	156.8	C_5	198.0	C_6	103.9
C_0	68.5	C_1	83.2	C_2	62.6	C_3	75.8	C_{4a}	63.6	C_{4b}	63.9	C_5	165.3	C_6	107.5
C_0	58.8	C_1	72.8	C_2	78.6	C_3	61.2	C_{4a}	53.8	C_{4b}	36.9	C_5	58.8	C_6	58.0
		C_1	25.2	C_2	29.7	C_3	58.9	C_{4a}	76.7	C_{4b}	103.9	C_5	82.7	C_6	185.2
		C_1	74.1	C_2	68.2	C_3	111.7	C_{4a}	52.7	C_{4b}	98.1	C_5	91.8	C_6	51.1
						C_3	39.6	C_{4a}	61.7						
								C_{4a}	39.6						
								C_{4a}	64.5						
Median	**44.1**	**Median**	**70.0**	**Median**	**79.2**	**Median**	**64.5**	**Median**	**61.7**	**Median**	**101.0**	**Median**	**88.6**	**Median**	**80.6**
LQ	**33.4**	**LQ**	**49.7**	**LQ**	**64.0**	**LQ**	**58.8**	**LQ**	**52.7**	**LQ**	**70.3**	**LQ**	**82.8**	**LQ**	**59.4**
UQ	**57.8**	**UQ**	**75.8**	**UQ**	**82.1**	**UQ**	**101.9**	**UQ**	**76.7**	**UQ**	**146.6**	**UQ**	**105.0**	**UQ**	**106.6**

Body Mass Index (Kg/m^2) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	18.5	C_1	20.4	C_2	20.4	C_3	29.1	C_{4a}	20.4	C_{4b}	39.8	C_5	25.9	C_6	29.1
C_0	18.5	C_1	20.4	C_2	28.5	C_3	25.9	C_{4a}	22.8	C_{4b}	24.5	C_5	28.5	C_6	28.5
C_0	23.4	C_1	24.8	C_2	25.5	C_3	25.5	C_{4a}	29.6	C_{4b}	24.5	C_5	28.5	C_6	23.1
C_0	23.4	C_1	24.8	C_2	24.4	C_3	21.1	C_{4a}	21.5	C_{4b}	28.2	C_5	24.8	C_6	23.1
C_0	19.2	C_1	24.8	C_2	24.4	C_3	26.0	C_{4a}	22.1	C_{4b}	22.1	C_5	22.8	C_6	28.2
C_0	19.2	C_1	21.1	C_2	21.5	C_3	26.0	C_{4a}	26.5	C_{4b}	38.0	C_5	29.6	C_6	26.5
C_0	26.0	C_1	19.4	C_2	27.5	C_3	39.8	C_{4a}	26.0	C_{4b}	29.2	C_5	39.8	C_6	26.0
C_0	26.0	C_1	19.4	C_2	29.2	C_3	39.8	C_{4a}	23.2	C_{4b}	21.9	C_5	25.0	C_6	23.2
		C_1	27.6	C_2	21.9	C_3	27.6	C_{4a}	26.3	C_{4b}	39.7	C_5	36.2	C_6	38.0
		C_1	27.1	C_2	22.1	C_3	27.1	C_{4a}	26.3	C_{4b}	39.7	C_5	25.0	C_6	27.5
						C_3	36.2	C_{4a}	21.0						
								C_{4a}	21.0						
								C_{4a}	22.1						
Median	**21.3**	**Median**	**23.0**	**Median**	**24.4**	**Median**	**27.1**	**Median**	**22.8**	**Median**	**28.7**	**Median**	**27.2**	**Median**	**27.0**
LQ	**19.0**	**LQ**	**20.4**	**LQ**	**21.9**	**LQ**	**25.9**	**LQ**	**21.5**	**LQ**	**24.5**	**LQ**	**25.0**	**LQ**	**23.9**
UQ	**24.1**	**UQ**	**24.8**	**UQ**	**27.0**	**UQ**	**32.7**	**UQ**	**26.3**	**UQ**	**39.3**	**UQ**	**29.3**	**UQ**	**28.4**

Duplex-derived Reflux Fraction at sites of measurements in Superficial Vein Segments only (cm^2) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.1	C_1	0.1	C_2	1.9	C_3	3.2	C_{4a}	2.2	C_{4b}	2.4	C_5	2.9	C_6	3.3
C_0	0.1	C_1	0.1	C_2	3.4	C_3	3.3	C_{4a}	5.4	C_{4b}	3.3	C_5	1.9	C_6	2.6
C_0	0.2	C_1	0.1	C_2	2.0	C_3	3.1	C_{4a}	4.5	C_{4b}	2.9	C_5	1.6	C_6	4.4
C_0	0.1	C_1	0.1	C_2	2.2	C_3	4.7	C_{4a}	1.5	C_{4b}	1.6	C_5	3.5	C_6	5.7
C_0	0.2	C_1	0.1	C_2	3.7	C_3	3.1	C_{4a}	2.0	C_{4b}	8.7	C_5	1.7	C_6	5.8
C_0	0.2	C_1	0.0	C_2	3.7	C_3	3.5	C_{4a}	2.9	C_{4b}	3.6	C_5	1.9	C_6	2.1
C_0	0.1	C_1	0.1	C_2	3.0	C_3	2.6	C_{4a}	4.0	C_{4b}	5.8	C_5	1.4	C_6	5.1
C_0	0.1	C_1	0.1	C_2	1.7	C_3	3.4	C_{4a}	2.9	C_{4b}	1.5	C_5	2.0	C_6	2.0
C_0	0.2	C_1	0.0	C_2	16.2	C_3	6.5	C_{4a}	5.6	C_{4b}	3.5	C_5	3.6	C_6	2.5
C_0	0.1	C_1	0.0	C_2	12.9	C_3	2.5					C_5	3.3		
C_0	0.2	C_1	0.0	C_2	2.8							C_5	3.1		
C_0	0.1	C_1	0.0												
C_0	0.2	C_1	0.2												
C_0	0.1	C_1	0.2												
C_0	0.3	C_1	0.0												
C_0	0.3	C_1	0.2												
		C_1	0.2												
		C_1	0.1												
		C_1	0.1												
		C_1	0.1												
		C_1	0.2												
Median	0.2	Median	0.1	Median	3.0	Median	3.2	Median	2.9	Median	3.3	Median	2.0	Median	3.3
LQ	0.1	LQ	0.0	LQ	2.1	LQ	3.1	LQ	2.2	LQ	2.4	LQ	1.8	LQ	2.5
UQ	0.2	UQ	0.1	UQ	3.7	UQ	3.5	UQ	4.5	UQ	3.6	UQ	3.2	UQ	5.1

Duplex-derived Reflux Volume Flow at sites of measurements in Superficial Vein Segments only (ml) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.0	C_1	0.1	C_2	0.6	C_3	5.5	C_{4a}	1.7	C_{4b}	1.8	C_5	0.1	C_6	7.5
C_0	0.2	C_1	0.1	C_2	1.4	C_3	1.2	C_{4a}	6.5	C_{4b}	8.8	C_5		C_6	4.5
C_0	0.0	C_1	0.1	C_2	0.5	C_3	0.7	C_{4a}	10.9	C_{4b}	1.2	C_5	0.2	C_6	1.6
C_0	0.1	C_1	0.0	C_2	0.5	C_3	9.1	C_{4a}	11.4	C_{4b}	0.9	C_5	11.0	C_6	8.8
C_0	0.1	C_1	0.1	C_2	0.8	C_3	5.7	C_{4a}	1.0	C_{4b}	4.9	C_5	1.1	C_6	1.1
C_0	0.0	C_1	0.0	C_2	4.2	C_3	24.6	C_{4a}	6.7	C_{4b}	4.2	C_5	1.1	C_6	3.2
C_0	0.0	C_1	0.0	C_2	1.8	C_3	3.6	C_{4a}	1.1	C_{4b}	21.5	C_5	0.9	C_6	15.1
C_0	0.0	C_1	0.0	C_2	0.6	C_3	4.4	C_{4a}	1.0	C_{4b}	0.7	C_5	7.1	C_6	0.8
C_0	0.1	C_1	0.1	C_2	10.4	C_3	1.7	C_{4a}	3.4	C_{4b}	4.8	C_5	1.2	C_6	0.8
C_0	0.0	C_1	0.0	C_2	3.3	C_3	2.5					C_5	9.2		
C_0	0.0	C_1	0.2	C_2	0.4							C_5	1.4		
C_0	0.0	C_1	0.1												
C_0	0.0	C_1	0.0												
C_0	0.0	C_1	0.0												
C_0	0.1	C_1	0.0												
C_0	0.5	C_1	0.1												
		C_1	0.0												
		C_1	0.0												
		C_1	0.0												
		C_1	0.0												
		C_1	0.0												
Median	0.0	Median	0.0	Median	0.8	Median	4.0	Median	3.4	Median	4.2	Median	1.2	Median	3.2
LO	0.0	LO	0.0	LO	0.5	LO	1.9	LO	1.1	LO	1.2	LO	1.0	LO	1.1
UO	0.1	UO	0.1	UO	2.5	UO	5.7	UO	6.7	UO	4.9	UO	5.7	UO	7.5

Duplex-derived Peak Reflux Velocity Ratio at sites of measurements in Superficial Vein Segments only (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.4	C_1	0.3	C_2	0.8	C_3	2.1	C_{4a}	1.6	C_{4b}	1.1	C_5	1.8	C_6	1.9
C_0	1.8	C_1	0.2	C_2	2.8	C_3	0.7	C_{4a}	1.8	C_{4b}	1.0	C_5	1.4	C_6	2.1
C_0	0.3	C_1	0.2	C_2	0.7	C_3	0.8	C_{4a}	2.0	C_{4b}	0.9	C_5	0.7	C_6	0.3
C_0	0.1	C_1	0.3	C_2	0.6	C_3	2.4	C_{4a}	1.1	C_{4b}	0.9	C_5	1.1	C_6	3.8
C_0	0.8	C_1	0.8	C_2	0.5	C_3	2.0	C_{4a}	0.8	C_{4b}	1.8	C_5	0.8	C_6	1.4
C_0	0.8	C_1	0.3	C_2	1.1	C_3	2.2	C_{4a}	1.7	C_{4b}	2.3	C_5	1.2	C_6	2.6
C_0	0.3	C_1	0.2	C_2	1.7	C_3	1.9	C_{4a}	2.0	C_{4b}	3.0	C_5	1.5	C_6	1.8
C_0	1.0	C_1	0.2	C_2	0.6	C_3	1.4	C_{4a}	1.1	C_{4b}	1.6	C_5	1.5	C_6	0.5
C_0	0.2	C_1	0.2	C_2	2.0	C_3	2.1	C_{4a}	3.4	C_{4b}	1.7	C_5	2.6	C_6	1.8
C_0	0.2	C_1	0.2	C_2	3.1	C_3	2.4					C_5	1.1		
C_0	0.3	C_1	0.3	C_2	1.2							C_5	2.5		
C_0	0.1	C_1	0.2												
C_0	0.5	C_1	0.1												
C_0	1.9	C_1	0.5												
C_0	0.4	C_1	0.3												
C_0	0.3	C_1	0.2												
		C_1	0.2												
		C_1	0.4												
		C_1	0.2												
		C_1	0.6												
Median	0.3	Median	0.2	Median	1.1	Median	2.0	Median	1.7	Median	1.6	Median	1.4	Median	1.8
LQ	0.2	LQ	0.2	LQ	0.6	LQ	1.5	LQ	1.1	LQ	1.0	LQ	1.1	LQ	1.4
UQ	0.8	UQ	0.3	UQ	1.9	UQ	2.1	UQ	2.0	UQ	1.8	UQ	1.7	UQ	2.1

Duplex-derived Reflux Time Ratio at sites of measurements in Superficial Vein Segments only (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.4	C_1	0.5	C_2	3.2	C_3	3.3	C_{4a}	2.6	C_{4b}	2.4	C_5	3.8	C_6	2.5
C_0	0.6	C_1	0.3	C_2	3.7	C_3	3.9	C_{4a}	3.3	C_{4b}	3.6	C_5	1.9	C_6	2.2
C_0	0.3	C_1	0.2	C_2	2.2	C_3	10.5	C_{4a}	4.4	C_{4b}	1.8	C_5	1.4	C_6	7.8
C_0	0.3	C_1	0.3	C_2	3.7	C_3	3.6	C_{4a}	1.1	C_{4b}	2.3	C_5	3.0	C_6	3.0
C_0	0.4	C_1	0.2	C_2	3.9	C_3	3.3	C_{4a}	2.3	C_{4b}	11.0	C_5	1.7	C_6	4.6
C_0	0.2	C_1	0.2	C_2	2.1	C_3	3.3	C_{4a}	2.1	C_{4b}	1.5	C_5	2.6	C_6	1.8
C_0	0.3	C_1	0.4	C_2	3.0	C_3	5.1	C_{4a}	2.0	C_{4b}	6.6	C_5	1.2	C_6	2.7
C_0	0.6	C_1	0.4	C_2	4.1	C_3	3.8	C_{4a}	2.8	C_{4b}	1.4	C_5	1.6	C_6	2.3
C_0	0.3	C_1	0.4	C_2	9.1	C_3	7.1	C_{4a}	4.5	C_{4b}	3.7	C_5	2.4	C_6	2.4
C_0	0.1	C_1	0.3	C_2	10.3	C_3	2.6					C_5	3.6		
C_0	0.2	C_1	0.5	C_2	2.5							C_5	1.9		
C_0	0.2	C_1	0.6												
C_0	0.5	C_1	0.2												
C_0	0.7	C_1	0.4												
C_0	0.9	C_1	0.2												
C_0	1.5	C_1	0.5												
		C_1	0.4												
		C_1	0.6												
		C_1	0.2												
		C_1	1.3												
Median	0.3	Median	0.4	Median	3.7	Median	3.7	Median	2.6	Median	2.4	Median	1.9	Median	2.5
LQ	0.3	LQ	0.2	LQ	2.8	LQ	3.3	LQ	2.1	LQ	1.8	LQ	1.7	LQ	2.3
UQ	0.6	UQ	0.5	UQ	4.0	UQ	4.8	UQ	3.3	UQ	3.7	UQ	2.8	UQ	3.0

Duplex-derived Reflux Fraction at sites of measurements in Deep Vein Segments only (cm^2) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.1	C_1	0.1	C_2	0.1	C_3	0.5	C_{4a}	0.9	C_{4b}	1.2	C_5	0.7	C_6	0.3
C_0	0.1	C_1	0.1	C_2	0.1	C_3	1.4	C_{4a}	0.7	C_{4b}	0.7	C_5	0.8	C_6	0.9
C_0	0.0	C_1	0.0	C_2	0.2	C_3	2.0	C_{4a}	0.5	C_{4b}	1.6	C_5	0.7	C_6	0.9
C_0	0.1	C_1	0.1	C_2	0.1	C_3	1.2	C_{4a}	1.5	C_{4b}	1.2	C_5	0.3	C_6	0.7
C_0	0.1	C_1	0.1	C_2	0.1	C_3	0.7	C_{4a}	0.4	C_{4b}	2.7	C_5	1.2	C_6	0.4
C_0	0.1	C_1	0.1	C_2	0.1			C_{4a}	0.9	C_{4b}	0.7	C_5	0.9		
C_0	0.1	C_1	0.1	C_2	0.1			C_{4a}	0.7						
C_0	0.0	C_1	0.2	C_2	0.1			C_{4a}	0.5						
C_0	0.1	C_1	0.1	C_2	0.0										
C_0	0.1	C_1	0.1	C_2	0.1										
C_0	0.1	C_1	0.1	C_2	0.1										
C_0	0.1	C_1	0.1	C_2	0.1										
C_0	0.1	C_1	0.0	C_2	0.1										
C_0	0.1	C_1	0.1	C_2	0.0										
C_0	0.0	C_1	0.1	C_2	0.1										
C_0	0.0	C_1	0.1	C_2	0.1										
C_0	0.1	C_1	0.1	C_2	0.0										
C_0	0.1	C_1	0.1	C_2	0.1										
C_0	0.2	C_1	0.0	C_2	0.1										
C_0	0.0	C_1	0.1	C_2	0.1										
C_0	0.3	C_1	0.1	C_2	0.2										
C_0	0.3	C_1	0.1	C_2	0.0										
C_0	0.3	C_1	0.2	C_2	0.3										
C_0	0.3	C_1	0.0	C_2	0.3										
		C_1	0.3	C_2	0.3										
		C_1	0.3	C_2	0.1										
		C_1	0.3	C_2	0.2										
		C_1	0.1	C_2	0.1										
		C_1	0.2	C_2	0.1										
		C_1	0.0	C_2	0.1										
Median	**0.1**	**Median**	**0.1**	**Median**	**0.1**	**Median**	**1.2**	**Median**	**0.7**	**Median**	**1.2**	**Median**	**0.8**	**Median**	**0.7**
LQ	**0.1**	**LQ**	**0.1**	**LQ**	**0.1**	**LQ**	**0.7**	**LQ**	**0.5**	**LQ**	**0.8**	**LQ**	**0.7**	**LQ**	**0.4**
UQ	**0.1**	**UQ**	**0.1**	**UQ**	**0.1**	**UQ**	**1.4**	**UQ**	**0.9**	**UQ**	**1.5**	**UQ**	**0.9**	**UQ**	**0.9**

Duplex-derived Reflux Volume Flow at sites of measurements in Deep Vein Segments only (ml) (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.4	C_1	2.2	C_2	1.4	C_3	1.7	C_{4a}	4.9	C_{4b}	4.9	C_5	0.5	C_6	3.7
C_0	1.8	C_1	2.4	C_2	1.4	C_3	12.3	C_{4a}	5.8	C_{4b}	1.3	C_5	1.9	C_6	1.7
C_0	0.2	C_1	0.7	C_2	0.6	C_3	0.4	C_{4a}	2.6	C_{4b}	5.2	C_5	4.7	C_6	10.8
C_0	0.3	C_1	0.1	C_2	2.2	C_3	0.2	C_{4a}	20.7	C_{4b}	0.4	C_5	1.8	C_6	10.2
C_0	0.2	C_1	0.5	C_2	2.4	C_3	10.3	C_{4a}	27.0	C_{4b}	4.2	C_5	0.4	C_6	4.9
C_0	0.2	C_1	0.4	C_2	0.7			C_{4a}	5.4	C_{4b}	5.7	C_5	23.0		
C_0	1.3	C_1	0.2	C_2	0.1			C_{4a}	5.0						
C_0	2.9	C_1	0.4	C_2	0.5			C_{4a}	2.3						
C_0	0.4	C_1	0.9	C_2	0.4										
C_0	0.5	C_1	0.2	C_2	0.2										
C_0	0.5	C_1	0.3	C_2	0.4										
C_0	0.6	C_1	0.6	C_2	2.1										
C_0	0.2	C_1	0.5	C_2	0.9										
C_0	1.0	C_1	0.8	C_2	1.0										
C_0	1.7	C_1	0.2	C_2	1.1										
C_0	0.4	C_1	0.4	C_2	0.2										
C_0	0.1	C_1	0.6	C_2	0.1										
C_0	0.1	C_1	1.0	C_2	0.0										
C_0	0.0	C_1	1.1	C_2	0.0										
C_0	0.0	C_1	0.2	C_2	0.0										
C_0	0.0	C_1	0.1	C_2	0.0										
C_0	0.0	C_1	0.1	C_2	0.0										
C_0	0.0	C_1	0.0	C_2	0.1										
C_0	0.1	C_1	0.0	C_2	0.1										
		C_1	0.0	C_2	0.0										
		C_1	0.0	C_2	1.4										
		C_1	0.0	C_2	0.1										
		C_1	0.1	C_2	0.1										
		C_1	0.1	C_2	0.1										
		C_1	0.0	C_2	0.0										
Median	**0.3**	**Median**	**0.3**	**Median**	**0.2**	**Median**	**1.7**	**Median**	**5.2**	**Median**	**4.5**	**Median**	**1.9**	**Median**	**4.9**
LQ	**0.1**	**LQ**	**0.1**	**LQ**	**0.1**	**LQ**	**0.4**	**LQ**	**4.4**	**LQ**	**2.0**	**LQ**	**0.8**	**LQ**	**3.7**
UQ	**0.6**	**UQ**	**0.6**	**UQ**	**1.0**	**UQ**	**10.3**	**UQ**	**9.6**	**UQ**	**5.1**	**UQ**	**4.0**	**UQ**	**10.2**

Duplex-derived Peak Reflux Velocity Ratio at sites of measurements in Deep Vein Segments only (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)																	
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.1	C_1	0.2	C_2	0.1	C_3	1.0	C_{4a}	0.6	C_{4b}	0.8	C_5	1.2	C_6	0.4		
C_0	0.2	C_1	0.1	C_2	0.9	C_3	1.3	C_{4a}	0.2	C_{4b}	0.6	C_5	0.4	C_6	1.0		
C_0	0.1	C_1	0.2	C_2	0.5	C_3	1.5	C_{4a}	0.5	C_{4b}	1.5	C_5	1.0	C_6	0.9		
C_0	0.2	C_1	0.1	C_2	0.4	C_3	0.6	C_{4a}	0.6	C_{4b}	1.8	C_5	0.3	C_6	0.4		
C_0	0.1	C_1	0.2	C_2	0.1	C_3	0.6	C_{4a}	0.6	C_{4b}	1.0	C_5	1.8	C_6	0.3		
C_0	0.1	C_1	0.1	C_2	0.1			C_{4a}	0.3	C_{4b}	0.4	C_5	0.8				
C_0	0.1	C_1	0.2	C_2	0.8			C_{4a}	0.5								
C_0	0.1	C_1	0.1	C_2	0.5			C_{4a}	0.7								
C_0	0.3	C_1	0.1	C_2	0.7												
C_0	0.4	C_1	0.1	C_2	0.1												
C_0	0.2	C_1	0.1	C_2	0.1												
C_0	0.3	C_1	0.2	C_2	0.1												
C_0	0.2	C_1	0.1	C_2	0.1												
C_0	0.2	C_1	0.2	C_2	0.1												
C_0	0.2	C_1	0.1	C_2	0.2												
C_0	0.2	C_1	0.1	C_2	0.2												
C_0	0.2	C_1	0.2	C_2	0.1												
C_0	0.3	C_1	0.2	C_2	0.1												
C_0	0.3	C_1	0.1	C_2	0.4												
C_0	0.3	C_1	0.1	C_2	0.4												
C_0	0.1	C_1	0.1	C_2	0.8												
C_0	0.3	C_1	0.4	C_2	0.5												
C_0	2.3	C_1	0.8	C_2	0.4												
C_0	0.5	C_1	0.5	C_2	0.4												
C_0	0.5	C_1	0.4	C_2	0.4												
		C_1	0.4	C_2	0.6												
		C_1	0.4	C_2	0.3												
		C_1	0.3	C_2	0.4												
		C_1	0.1	C_2	0.1												
		C_1	0.4	C_2	0.4												
		C_1	0.2	C_2	0.2												
Median	**0.2**	**Median**	**0.2**	**Median**	**0.3**	**Median**	**1.0**	**Median**	**0.6**	**Median**	**0.9**	**Median**	**0.9**	**Median**	**0.4**		
LQ	**0.1**	**LQ**	**0.1**	**LQ**	**0.1**	**LQ**	**0.6**	**LQ**	**0.4**	**LQ**	**0.7**	**LQ**	**0.5**	**LQ**	**0.4**		
UQ	**0.3**	**UQ**	**0.3**	**UQ**	**0.4**	**UQ**	**1.3**	**UQ**	**0.6**	**UQ**	**1.4**	**UQ**	**1.2**	**UQ**	**0.9**		

Duplex-derived Reflux Time Ratio at sites of measurements in Deep Vein Segments only (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.4	C_1	0.5	C_2	0.5	C_3	0.7	C_{4a}	1.8	C_{4b}	2.5	C_5	1.0	C_6	0.8
C_0	0.2	C_1	0.4	C_2	0.1	C_3	1.7	C_{4a}	2.3	C_{4b}	1.2	C_5	1.4	C_6	1.0
C_0	0.1	C_1	0.2	C_2	1.0	C_3	2.0	C_{4a}	0.8	C_{4b}	2.4	C_5	1.0	C_6	1.8
C_0	0.3	C_1	0.2	C_2	0.4	C_3	2.0	C_{4a}	2.9	C_{4b}	1.4	C_5	1.3	C_6	1.7
C_0	0.3	C_1	0.2	C_2	0.2	C_3	1.0	C_{4a}	0.9	C_{4b}	2.8	C_5	1.6	C_6	1.3
C_0	0.4	C_1	0.2	C_2	0.2			C_{4a}	1.7	C_{4b}	1.9	C_5	1.5		
C_0	0.4	C_1	0.4	C_2	0.2			C_{4a}	1.5						
C_0	0.5	C_1	0.5	C_2	0.6			C_{4a}	0.9						
C_0	0.3	C_1	0.5	C_2	0.4										
C_0	0.4	C_1	0.1	C_2	0.8										
C_0	0.4	C_1	0.2	C_2	0.5										
C_0	0.4	C_1	0.4	C_2	0.4										
C_0	0.4	C_1	0.2	C_2	0.6										
C_0	0.6	C_1	0.2	C_2	0.4										
C_0	0.3	C_1	0.1	C_2	0.8										
C_0	0.3	C_1	0.2	C_2	0.5										
C_0	0.4	C_1	0.4	C_2	0.4										
C_0	0.4	C_1	0.4	C_2	0.3										
C_0	0.5	C_1	0.4	C_2	0.5										
C_0	0.2	C_1	0.1	C_2	0.5										
C_0	0.5	C_1	0.3	C_2	0.8										
C_0	0.2	C_1	0.1	C_2	0.5										
C_0	0.5	C_1	0.3	C_2	0.8										
C_0	0.7	C_1	0.3	C_2	0.9										
C_0	0.6	C_1	0.5	C_2	0.6										
C_0	0.8	C_1	0.5	C_2	0.4										
		C_1	0.8	C_2	0.6										
		C_1	1.1	C_2	0.4										
		C_1	0.6	C_2	0.8										
		C_1	0.4	C_2	0.5										
		C_1	0.4	C_2	0.4										
		C_1	0.3	C_2	0.2										
Median	0.4	Median	0.4	Median	0.5	Median	1.7	Median	1.6	Median	2.1	Median	1.4	Median	1.3
LQ	0.3	LQ	0.2	LQ	0.4	LQ	1.0	LQ	0.9	LQ	1.5	LQ	1.1	LQ	1.0
UQ	0.5	UQ	0.4	UQ	0.6	UQ	2.0	UQ	1.9	UQ	2.5	UQ	1.5	UQ	1.7

Tissue Tonometry Data for Distance Travelled (X_0) in mm during Initial Fast Indentation Phase I (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	2.5	C_1	3.6	C_2	3.0	C_3	2.5	C_{4a}	2.5	C_{4b}	0.9	C_5	0.9	C_6	2.1
C_0	3.1	C_1	2.8	C_2	2.5	C_3	3.6	C_{4a}	2.2	C_{4b}	0.8	C_5	1.0	C_6	1.0
C_0	2.6	C_1	2.6	C_2	3.8	C_3	2.6	C_{4a}	2.9	C_{4b}	0.7	C_5	1.3	C_6	0.9
C_0	2.6	C_1	2.5	C_2	2.8	C_3	2.1	C_{4a}	2.5	C_{4b}	1.0	C_5	1.1	C_6	1.0
C_0	3.4	C_1	2.3	C_2	3.0	C_3	2.9	C_{4a}	2.7	C_{4b}	1.2	C_5	1.0	C_6	1.0
C_0	3.7	C_1	3.5	C_2	3.0	C_3	3.4	C_{4a}	3.5	C_{4b}	1.5	C_5	1.2	C_6	1.1
C_0	2.5	C_1	3.6	C_2	3.5	C_3	2.3	C_{4a}	2.2	C_{4b}	1.5	C_5	0.8	C_6	1.6
C_0	2.6	C_1	2.3	C_2	2.9	C_3	2.8	C_{4a}	3.0	C_{4b}	0.7	C_5	1.9	C_6	0.5
		C_1	4.4	C_2	2.5	C_3	2.5	C_{4a}	2.9	C_{4b}	0.9	C_5	0.9	C_6	0.5
		C_1	4.2	C_2	2.3	C_3	3.6	C_{4a}	2.6	C_{4b}	1.1	C_5	0.7	C_6	0.9
						C_3	3.5	C_{4a}	2.9						
								C_{4a}	2.9						
								C_{4a}	3.6						
Median	2.6	Median	3.2	Median	2.9	Median	2.8	Median	2.9	Median	1.0	Median	1.0	Median	1.0
LQ	2.5	LQ	2.6	LQ	2.6	LQ	2.5	LQ	2.5	LQ	0.8	LQ	0.9	LQ	0.9
UQ	3.2	UQ	3.6	UQ	3.0	UQ	3.4	UQ	2.9	UQ	1.2	UQ	1.2	UQ	1.1

Tissue Tonometry Data for Distance Travelled (X_1-X_0) in mm during Slower Indentation Phase II (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.3	C_1	0.6	C_2	1.2	C_3	0.3	C_{4a}	0.3	C_{4b}	0.4	C_5	0.6	C_6	1.1
C_0	0.3	C_1	0.4	C_2	0.7	C_3	0.5	C_{4a}	0.4	C_{4b}	0.6	C_5	0.3	C_6	0.3
C_0	0.2	C_1	0.3	C_2	0.8	C_3	0.7	C_{4a}	0.9	C_{4b}	1.0	C_5	0.3	C_6	0.6
C_0	0.2	C_1	0.3	C_2	0.3	C_3	0.5	C_{4a}	1.0	C_{4b}	0.7	C_5	0.8	C_6	1.1
C_0	0.3	C_1	0.3	C_2	0.4	C_3	0.6	C_{4a}	0.6	C_{4b}	0.9	C_5	0.1	C_6	0.4
C_0	0.3	C_1	0.3	C_2	0.4	C_3	0.5	C_{4a}	0.8	C_{4b}	1.2	C_5	1.1	C_6	0.5
C_0	0.2	C_1	0.6	C_2	0.3	C_3	0.2	C_{4a}	0.3	C_{4b}	0.9	C_5	0.5	C_6	1.4
C_0	0.3	C_1	0.1	C_2	0.5	C_3	0.6	C_{4a}	0.5	C_{4b}	0.4	C_5	1.2	C_6	0.6
		C_1	0.2	C_2	0.4	C_3	0.6	C_{4a}	0.7	C_{4b}	0.7	C_5	0.3	C_6	0.2
		C_1	0.1	C_2	0.4	C_3	0.8	C_{4a}	0.7	C_{4b}	0.7	C_5	0.5	C_6	0.5
						C_3	0.5	C_{4a}	0.6						
								C_{4a}	0.7						
								C_{4a}	0.9						
Median	0.3	Median	0.3	Median	0.4	Median	0.5	Median	0.7	Median	0.7	Median	0.5	Median	0.5
LQ	0.2	LQ	0.2	LQ	0.4	LQ	0.5	LQ	0.5	LQ	0.6	LQ	0.3	LQ	0.4
UQ	0.3	UQ	0.4	UQ	0.7	UQ	0.6	UQ	0.8	UQ	0.9	UQ	0.8	UQ	0.9

Tissue Tonometry Data for Rate Constant Parameter (-1/Tau) in secs^{-1} during Slow Indentation Phase II (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)																
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	
C_0	2.5	C_1	2.3	C_2	1.8	C_3	1.9	C_{4a}	3.1	C_{4b}	4.0	C_5	1.6	C_6	2.3	
C_0	3.0	C_1	2.2	C_2	2.4	C_3	3.3	C_{4a}	3.1	C_{4b}	2.0	C_5	2.6	C_6	3.1	
C_0	3.0	C_1	2.9	C_2	2.1	C_3	3.9	C_{4a}	1.9	C_{4b}	5.1	C_5	2.4	C_6	3.3	
C_0	1.9	C_1	2.1	C_2	3.1	C_3	4.1	C_{4a}	4.2	C_{4b}	2.3	C_5	4.1	C_6	2.8	
C_0	2.7	C_1	2.1	C_2	2.9	C_3	2.7	C_{4a}	2.7	C_{4b}	5.1	C_5	4.6	C_6	3.7	
C_0	2.6	C_1	3.0	C_2	3.7	C_3	3.0	C_{4a}	4.9	C_{4b}	2.2	C_5	3.6	C_6	4.3	
C_0	2.1	C_1	2.7	C_2	3.4	C_3	6.4	C_{4a}	3.5	C_{4b}	3.3	C_5	2.3	C_6	5.3	
C_0	2.3	C_1	1.8	C_2	5.3	C_3	4.2	C_{4a}	6.3	C_{4b}	2.0	C_5	1.9	C_6	2.4	
		C_1	3.1	C_2	2.0	C_3	2.7	C_{4a}	3.0	C_{4b}	3.8	C_5	2.2	C_6	2.2	
		C_1	5.1	C_2	2.1	C_3	4.7	C_{4a}	2.0	C_{4b}	5.2	C_5	3.3	C_6	3.3	
						C_3	2.9	C_{4a}	8.6							
								C_{4a}	4.4							
								C_{4a}	2.5							
Median	2.5	Median	2.5	Median	2.7	Median	3.3	Median	3.1	Median	3.5	Median	2.5	Median	3.2	
LO	2.3	LO	2.1	LO	2.1	LO	2.8	LO	2.7	LO	2.2	LO	2.2	LO	2.5	
UO	2.7	UO	3.0	UO	3.4	UO	4.2	UO	4.4	UO	4.8	UO	3.5	UO	3.6	

Tissue Tonometry Data for Distance Travelled (X_1-X_0) in mm during Slower Indentation Phase III (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)																
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	
C_0	0.2	C_1	0.3	C_2	0.2	C_3	0.5	C_{4a}	0.9	C_{4b}	0.6	C_5	0.3	C_6	2.3	
C_0	0.2	C_1	0.0	C_2	0.9	C_3	0.5	C_{4a}	0.5	C_{4b}	0.1	C_5	0.0	C_6	1.0	
C_0	0.3	C_1	0.1	C_2	0.8	C_3	0.7	C_{4a}	0.8	C_{4b}	1.3	C_5	0.1	C_6	0.6	
C_0	0.3	C_1	0.2	C_2	0.3	C_3	0.4	C_{4a}	0.9	C_{4b}	0.5	C_5	0.8	C_6	0.9	
C_0	0.1	C_1	0.2	C_2	0.4	C_3	0.5	C_{4a}	0.6	C_{4b}	0.6	C_5	0.0	C_6	0.2	
C_0	0.1	C_1	0.3	C_2	0.4	C_3	0.3	C_{4a}	0.7	C_{4b}	0.7	C_5	1.5	C_6	0.3	
C_0	0.3	C_1	0.2	C_2	0.3	C_3	0.5	C_{4a}	0.3	C_{4b}	0.8	C_5	0.1	C_6	1.6	
C_0	0.1	C_1	0.0	C_2	0.4	C_3	0.5	C_{4a}	0.8	C_{4b}	0.2	C_5	0.8	C_6	0.7	
		C_1	0.1	C_2	0.2	C_3	0.3	C_{4a}	0.2	C_{4b}	0.1	C_5	0.2	C_6	0.3	
		C_1	0.1	C_2	0.0	C_3	0.0	C_{4a}	0.7	C_{4b}	0.1	C_5	0.1	C_6	0.4	
						C_3	0.3	C_{4a}	1.1							
								C_{4a}	0.8							
								C_{4a}	0.7							
Median	0.2	Median	0.2	Median	0.3	Median	0.5	Median	0.7	Median	0.5	Median	0.2	Median	0.7	
LO	0.1	LO	0.1	LO	0.2	LO	0.3	LO	0.6	LO	0.1	LO	0.1	LO	0.3	
UO	0.3	UO	0.2	UO	0.4	UO	0.5	UO	0.8	UO	0.6	UO	0.7	UO	1.0	

Tissue Tonometry Data for Rate Constant Parameter (-1/Tau) in $secs^{-1}$ during Slow Indentation Phase III (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	45.7	C_1	59.4	C_2	179.7	C_3	70.8	C_{4a}	39.1	C_{4b}	28.1	C_5	52.2	C_6	52.4
C_0	54.3	C_1	495.9	C_2	62.0	C_3	175.2	C_{4a}	59.6	C_{4b}	69.5	C_5	61.1	C_6	125.4
C_0	325.8	C_1	82.9	C_2	58.8	C_3	89.9	C_{4a}	71.6	C_{4b}	195.5	C_5	44.4	C_6	67.9
C_0	57.5	C_1	67.9	C_2	81.8	C_3	108.2	C_{4a}	77.7	C_{4b}	144.1	C_5	321.6	C_6	66.8
C_0	536.5	C_1	211.6	C_2	108.1	C_3	89.6	C_{4a}	61.0	C_{4b}	54.3	C_5	475.6	C_6	74.1
C_0	86.2	C_1	68.8	C_2	65.7	C_3	240.0	C_{4a}	55.7	C_{4b}	49.9	C_5	82.1	C_6	33.0
C_0	101.5	C_1	161.5	C_2	76.8	C_3	42.6	C_{4a}	49.5	C_{4b}	42.4	C_5	692.5	C_6	118.8
C_0	64.3	C_1	93.4	C_2	78.3	C_3	101.0	C_{4a}	64.7	C_{4b}	153.8	C_5	43.9	C_6	101.2
		C_1	903.5	C_2	132.2	C_3	71.1	C_{4a}	137.1	C_{4b}	58.5	C_5	653.2	C_6	129.9
		C_1	1602.6	C_2	135.8	C_3	88.9	C_{4a}	72.7	C_{4b}	54.7	C_5	80.3	C_6	47.8
						C_3	81.6	C_{4a}	223.7						
								C_{4a}	56.0						
								C_{4a}	50.2						
Median	**75.3**	**Median**	**127.4**	**Median**	**80.1**	**Median**	**89.6**	**Median**	**50.2**	**Median**	**56.6**	**Median**	**81.2**	**Median**	**71.0**
LO	**56.7**	**LO**	**72.3**	**LO**	**68.4**	**LO**	**76.4**	**LO**	**61.0**	**LO**	**51.0**	**LO**	**54.4**	**LO**	**56.0**
UO	**157.6**	**UO**	**424.8**	**UO**	**126.2**	**UO**	**104.6**	**UO**	**55.7**	**UO**	**125.5**	**UO**	**437.1**	**UO**	**114.4**

Tissue Tonometry Data for Series Hooke Element (Spring Constant) C_2 in g/mm during Phase I (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	1.3	C_1	0.9	C_2	1.1	C_3	1.3	C_{4a}	1.3	C_{4b}	3.8	C_5	3.6	C_6	1.6
C_0	1.1	C_1	1.2	C_2	1.3	C_3	0.9	C_{4a}	1.5	C_{4b}	4.2	C_5	3.3	C_6	3.3
C_0	1.3	C_1	1.3	C_2	0.9	C_3	1.3	C_{4a}	1.1	C_{4b}	4.5	C_5	2.5	C_6	3.7
C_0	1.3	C_1	1.3	C_2	1.2	C_3	1.6	C_{4a}	1.3	C_{4b}	3.2	C_5	3.1	C_6	3.3
C_0	1.0	C_1	1.4	C_2	1.1	C_3	1.1	C_{4a}	1.2	C_{4b}	2.7	C_5	3.3	C_6	3.4
C_0	0.9	C_1	0.9	C_2	1.1	C_3	1.0	C_{4a}	1.0	C_{4b}	2.1	C_5	2.7	C_6	3.0
C_0	1.3	C_1	0.9	C_2	0.9	C_3	1.4	C_{4a}	1.5	C_{4b}	2.3	C_5	4.0	C_6	2.1
C_0	1.3	C_1	1.4	C_2	1.1	C_3	1.2	C_{4a}	1.1	C_{4b}	5.0	C_5	1.8	C_6	7.1
		C_1	0.8	C_2	1.3	C_3	1.3	C_{4a}	1.1	C_{4b}	3.7	C_5	3.6	C_6	6.0
		C_1	0.8	C_2	1.4	C_3	0.9	C_{4a}	1.3	C_{4b}	2.9	C_5	4.7	C_6	3.5
						C_3	1.0	C_{4a}	1.2						
								C_{4a}	1.1						
								C_{4a}	0.9						
Median	**1.3**	**Median**	**1.1**	**Median**	**1.1**	**Median**	**1.2**	**Median**	**1.2**	**Median**	**3.5**	**Median**	**3.3**	**Median**	**3.4**
LO	**1.1**	**LO**	**0.9**	**LO**	**1.1**	**LO**	**1.0**	**LO**	**1.1**	**LO**	**2.8**	**LO**	**2.8**	**LO**	**3.1**
UO	**1.3**	**UO**	**1.3**	**UO**	**1.3**	**UO**	**1.3**	**UO**	**1.3**	**UO**	**4.1**	**UO**	**3.6**	**UO**	**3.7**

Tissue Tonometry Data for Series Kelvin Element (Spring Constant) C_i in g/mm during Phase II (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	10.1	C_1	5.8	C_2	2.7	C_3	12.7	C_{4a}	11.3	C_{4b}	7.7	C_5	5.2	C_6	3.0
C_0	9.6	C_1	7.5	C_2	4.8	C_3	7.3	C_{4a}	8.2	C_{4b}	5.3	C_5	10.0	C_6	12.7
C_0	18.9	C_1	10.6	C_2	4.1	C_3	4.9	C_{4a}	3.6	C_{4b}	3.5	C_5	10.0	C_6	5.3
C_0	13.9	C_1	9.7	C_2	9.8	C_3	7.3	C_{4a}	3.3	C_{4b}	4.6	C_5	4.1	C_6	3.1
C_0	12.2	C_1	10.0	C_2	8.8	C_3	5.2	C_{4a}	5.4	C_{4b}	3.5	C_5	31.8	C_6	9.4
C_0	11.8	C_1	12.5	C_2	9.5	C_3	6.5	C_{4a}	4.0	C_{4b}	2.8	C_5	3.0	C_6	6.9
C_0	19.6	C_1	5.3	C_2	13.0	C_3	16.4	C_{4a}	10.6	C_{4b}	3.6	C_5	6.9	C_6	2.3
C_0	10.8	C_1	27.4	C_2	6.3	C_3	6.0	C_{4a}	6.2	C_{4b}	8.7	C_5	2.8	C_6	5.5
		C_1	19.3	C_2	7.6	C_3	5.3	C_{4a}	4.4	C_{4b}	4.4	C_5	11.5	C_6	13.5
		C_1	26.1	C_2	7.8	C_3	4.3	C_{4a}	4.9	C_{4b}	4.5	C_5	7.3	C_6	6.9
						C_3	6.3	C_{4a}	5.6						
								C_{4a}	4.9						
								C_{4a}	3.5						
Median	**12.0**	**Median**	**10.3**	**Median**	**7.7**	**Median**	**6.3**	**Median**	**4.9**	**Median**	**4.5**	**Median**	**7.1**	**Median**	**6.2**
LQ	**10.6**	**LQ**	**8.0**	**LQ**	**5.2**	**LQ**	**5.2**	**LQ**	**4.0**	**LQ**	**3.5**	**LQ**	**4.4**	**LQ**	**3.7**
UQ	**15.2**	**UQ**	**17.6**	**UQ**	**9.3**	**UQ**	**7.3**	**UQ**	**6.2**	**UQ**	**5.1**	**UQ**	**10.0**	**UQ**	**8.8**

Tissue Tonometry Data for Dashpot Constant, k , in g/(mm/sec) during Phase II (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	25.6	C_1	13.2	C_2	4.8	C_3	24.0	C_{4a}	35.0	C_{4b}	30.7	C_5	8.5	C_6	6.9
C_0	28.5	C_1	16.7	C_2	11.5	C_3	24.1	C_{4a}	25.2	C_{4b}	10.5	C_5	26.3	C_6	38.8
C_0	56.6	C_1	30.2	C_2	8.8	C_3	19.1	C_{4a}	6.8	C_{4b}	17.6	C_5	23.6	C_6	17.2
C_0	26.5	C_1	20.0	C_2	29.9	C_3	30.0	C_{4a}	13.8	C_{4b}	10.6	C_5	16.5	C_6	8.7
C_0	32.3	C_1	20.6	C_2	25.7	C_3	13.8	C_{4a}	14.7	C_{4b}	18.0	C_5	145.5	C_6	35.1
C_0	29.9	C_1	37.2	C_2	35.2	C_3	19.4	C_{4a}	19.8	C_{4b}	6.2	C_5	10.8	C_6	29.2
C_0	41.2	C_1	14.2	C_2	44.7	C_3	104.6	C_{4a}	36.9	C_{4b}	11.8	C_5	15.6	C_6	12.2
C_0	24.8	C_1	49.8	C_2	33.3	C_3	25.1	C_{4a}	39.2	C_{4b}	16.9	C_5	5.2	C_6	13.0
		C_1	60.2	C_2	14.9	C_3	14.2	C_{4a}	13.3	C_{4b}	16.7	C_5	25.4	C_6	29.1
		C_1	132.9	C_2	16.1	C_3	20.5	C_{4a}	9.8	C_{4b}	23.3	C_5	24.3	C_6	22.6
						C_3	18.6	C_{4a}	47.9						
								C_{4a}	21.8						
								C_{4a}	9.0						
Median	**29.2**	**Median**	**25.4**	**Median**	**20.9**	**Median**	**20.5**	**Median**	**19.8**	**Median**	**16.8**	**Median**	**20.1**	**Median**	**19.9**
LQ	**26.3**	**LQ**	**17.5**	**LQ**	**12.4**	**LQ**	**18.9**	**LQ**	**13.3**	**LQ**	**10.9**	**LQ**	**12.0**	**LQ**	**12.4**
UQ	**34.5**	**UQ**	**46.7**	**UQ**	**32.5**	**UQ**	**24.6**	**UQ**	**35.0**	**UQ**	**17.9**	**UQ**	**25.1**	**UQ**	**29.2**

Tissue Tonometry Data for Series Kelvin Element (Spring Constant) C_1 in g/mm during Phase III (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	16.7	C_1	10.0	C_2	13.7	C_3	7.3	C_{4a}	3.7	C_{4b}	6.0	C_5	9.7	C_6	1.5
C_0	18.6	C_1	328.8	C_2	3.7	C_3	6.2	C_{4a}	6.5	C_{4b}	29.9	C_5	109.6	C_6	3.3
C_0	11.9	C_1	27.8	C_2	4.4	C_3	4.8	C_{4a}	4.1	C_{4b}	2.5	C_5	23.5	C_6	5.8
C_0	10.7	C_1	14.8	C_2	12.8	C_3	8.2	C_{4a}	3.8	C_{4b}	6.5	C_5	4.1	C_6	3.5
C_0	31.6	C_1	19.9	C_2	8.5	C_3	7.1	C_{4a}	5.6	C_{4b}	5.4	C_5	402.6	C_6	15.7
C_0	44.2	C_1	10.7	C_2	8.6	C_3	10.1	C_{4a}	5.1	C_{4b}	5.1	C_5	2.3	C_6	12.7
C_0	12.0	C_1	18.2	C_2	11.2	C_3	6.6	C_{4a}	13.2	C_{4b}	4.3	C_5	45.1	C_6	2.0
C_0	52.0	C_1	420.5	C_2	7.5	C_3	6.6	C_{4a}	4.3	C_{4b}	18.1	C_5	4.1	C_6	4.5
		C_1	24.5	C_2	15.4	C_3	10.2	C_{4a}	14.9	C_{4b}	27.4	C_5	18.3	C_6	10.4
		C_1	49.0	C_2	164.4	C_3	89.5	C_{4a}	4.8	C_{4b}	25.3	C_5	32.1	C_6	7.7
						C_3	13.1	C_{4a}	2.9						
								C_{4a}	4.0						
								C_{4a}	5.0						
Median	**17.6**	**Median**	**22.2**	**Median**	**9.9**	**Median**	**7.3**	**Median**	**4.8**	**Median**	**6.2**	**Median**	**20.9**	**Median**	**5.2**
LQ	**11.9**	**LQ**	**15.7**	**LQ**	**7.8**	**LQ**	**6.6**	**LQ**	**4.0**	**LQ**	**5.2**	**LQ**	**5.5**	**LQ**	**3.3**
UQ	**34.8**	**UQ**	**43.7**	**UQ**	**13.5**	**UQ**	**10.2**	**UQ**	**5.6**	**UQ**	**23.5**	**UQ**	**41.8**	**UQ**	**9.7**

Tissue Tonometry Data for Dashpot Constant, k , in g/(mm/sec) during Phase III (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	762.0	C_1	591.9	C_2	2462.1	C_3	517.4	C_{4a}	142.9	C_{4b}	168.0	C_5	506.5	C_6	75.9
C_0	1008.3	C_1	163062.7	C_2	229.1	C_3	1087.0	C_{4a}	384.3	C_{4b}	2077.6	C_5	6697.0	C_6	408.3
C_0	3878.5	C_1	2306.2	C_2	257.8	C_3	434.7	C_{4a}	292.3	C_{4b}	490.7	C_5	1042.8	C_6	393.1
C_0	612.2	C_1	1006.2	C_2	1046.6	C_3	885.8	C_{4a}	294.4	C_{4b}	929.1	C_5	1311.0	C_6	235.6
C_0	16954.0	C_1	4200.4	C_2	921.1	C_3	639.6	C_{4a}	340.0	C_{4b}	292.7	C_5	191442.9	C_6	1160.3
C_0	3815.1	C_1	734.5	C_2	565.1	C_3	2426.3	C_{4a}	281.8	C_{4b}	253.7	C_5	186.4	C_6	417.4
C_0	1213.7	C_1	2932.2	C_2	858.2	C_3	280.2	C_{4a}	653.3	C_{4b}	180.8	C_5	31199.1	C_6	239.7
C_0	3344.5	C_1	39253.0	C_2	587.9	C_3	664.2	C_{4a}	276.3	C_{4b}	2786.1	C_5	179.5	C_6	455.9
		C_1	22112.2	C_2	2037.8	C_3	727.9	C_{4a}	2043.6	C_{4b}	1603.0	C_5	11933.2	C_6	1355.8
		C_1	78446.6	C_2	22327.0	C_3	7950.5	C_{4a}	345.5	C_{4b}	1383.6	C_5	2579.1	C_6	365.8
						C_3	1065.7	C_{4a}	645.2						
								C_{4a}	224.6						
								C_{4a}	250.1						
Median	**2279.1**	**Median**	**3566.3**	**Median**	**889.7**	**Median**	**727.9**	**Median**	**294.4**	**Median**	**709.9**	**Median**	**1945.1**	**Median**	**400.7**
LQ	**946.8**	**LQ**	**1331.2**	**LQ**	**570.8**	**LQ**	**578.5**	**LQ**	**276.3**	**LQ**	**263.5**	**LQ**	**640.6**	**LQ**	**271.2**
UQ	**3830.9**	**UQ**	**34967.8**	**UQ**	**1790.0**	**UQ**	**1076.4**	**UQ**	**384.3**	**UQ**	**1548.2**	**UQ**	**10624.1**	**UQ**	**446.2**

Duplex-derived Total Valve Closing Times in seconds in all incompetent vein segments examined (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.3	C_1	0.3	C_2	2.5	C_3	3.7	C_{4a}	3.0	C_{4b}	2.4	C_5	3.8	C_6	2.2
C_0	0.4	C_1	0.2	C_2	3.8	C_3	2.7	C_{4a}	2.8	C_{4b}	4.4	C_5	2.6	C_6	6.3
C_0	0.2	C_1	0.1	C_2	1.6	C_3	4.5	C_{4a}	4.1	C_{4b}	2.2	C_5	1.1	C_6	2.3
C_0	0.3	C_1	0.1	C_2	2.8	C_3	4.2	C_{4a}	1.1	C_{4b}	1.6	C_5	2.2	C_6	2.0
C_0	0.2	C_1	0.1	C_2	3.1	C_3	2.7	C_{4a}	1.9	C_{4b}	4.2	C_5	1.9	C_6	2.6
C_0	0.1	C_1	0.1	C_2	2.1	C_3	3.5	C_{4a}	1.7	C_{4b}	1.2	C_5	2.2	C_6	2.1
C_0	0.1	C_1	0.2	C_2	2.8	C_3	5.7	C_{4a}	1.7	C_{4b}	5.2	C_5	2.1	C_6	2.8
C_0	0.2	C_1	0.3	C_2	3.9	C_3	3.2	C_{4a}	1.1	C_{4b}	2.0	C_5	1.2	C_6	2.2
C_0	0.2	C_1	0.3	C_2	6.1	C_3	1.6	C_{4a}	3.9	C_{4b}	1.6	C_5	1.9	C_6	0.9
C_0	0.1	C_1	0.2	C_2	6.5	C_3	3.6	C_{4a}	1.8	C_{4b}	2.8	C_5	1.7	C_6	1.1
C_0	0.1	C_1	0.2	C_2	2.0	C_3	0.6	C_{4a}	2.6	C_{4b}	1.5	C_5	0.9	C_6	2.0
C_0	0.1	C_1	0.2			C_3	1.3	C_{4a}	1.2	C_{4b}	1.6	C_5	1.0	C_6	2.3
C_0	0.1	C_1	0.1			C_3	1.7	C_{4a}	2.9	C_{4b}	0.6	C_5	1.3	C_6	2.2
C_0	0.2	C_1	0.1			C_3	1.0	C_{4a}	0.9	C_{4b}	3.0	C_5	0.9	C_6	1.0
C_0	0.3	C_1	0.1			C_3	1.0	C_{4a}	1.7	C_{4b}	2.3	C_5	1.4		
C_0	0.3	C_1	0.3					C_{4a}	1.7			C_5	0.7		
C_0	0.5	C_1	0.2					C_{4a}	1.0			C_5	1.9		
C_0	0.2	C_1	0.4												
C_0	0.1	C_1	0.1												
C_0	0.3	C_1	0.4												
C_0	0.2	C_1	0.5												
C_0	0.3	C_1	0.3												
C_0	0.2	C_1	0.2												
C_0	0.3	C_1	0.2												
C_0	0.2	C_1	0.2												
C_0	0.3	C_1	0.2												
C_0	0.4	C_1	0.4												
C_0	0.4	C_1	0.5												
C_0	0.3	C_1	0.5												
C_0	0.4	C_1	0.1												
C_0	0.2	C_1	0.2												
C_0	0.2	C_1	0.3												
C_0	0.3	C_1	0.2												
C_0	0.3	C_1	0.2												
C_0	0.2	C_1	0.1												
C_0	0.1	C_1	0.2												
C_0	0.2	C_1	0.4												
C_0	0.2	C_1	0.4												
C_0	0.2	C_1	0.4												
C_0	0.3	C_1	0.2												
		C_1	0.2												

Duplex-derived Total Valve Closing Times in seconds in all incompetent vein segments examined (Data with Median values and Lower-Quartile / Upper-Quartile Ranges) continued.....															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
		C_1	0.2												
		C_1	0.2												
		C_1	0.3												
		C_1	0.3												
		C_1	0.3												
		C_1	0.2												
		C_1	0.1												
		C_1	0.1												
		C_1	0.2												
Median	0.2	Median	0.2	Median	2.8	Median	2.7	Median	1.7	Median	2.2	Median	1.7	Median	2.2
LQ	0.2	LQ	0.2	LQ	2.3	LQ	1.5	LQ	1.2	LQ	1.6	LQ	1.1	LQ	2.0
UQ	0.3	UQ	0.3	UQ	3.8	UQ	3.6	UQ	2.8	UQ	2.9	UQ	2.1	UQ	2.3

Duplex-derived incompetent Superficial vein diameters in mm (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	3.0	C_1	3.4	C_2	5.3	C_3	8.0	C_{4a}	4.3	C_{4b}	4.0	C_5	6.0	C_6	6.3
C_0	4.1	C_1	4.0	C_2	4.0	C_3	4.4	C_{4a}	5.2	C_{4b}	5.3	C_5	7.3	C_6	6.2
C_0	3.5	C_1	4.0	C_2	4.4	C_3	8.1	C_{4a}	7.0	C_{4b}	4.2	C_5	5.0	C_6	13.0
C_0	3.8	C_1	2.9	C_2	3.4	C_3	5.5	C_{4a}	10.5	C_{4b}	5.0	C_5	10.2	C_6	6.0
C_0	2.6	C_1	3.2	C_2	4.0	C_3	4.3	C_{4a}	9.0	C_{4b}	5.0	C_5	4.1	C_6	4.0
C_0	2.9	C_1	4.0	C_2	8.0	C_3	6.8	C_{4a}	8.0	C_{4b}	8.1	C_5	8.2	C_6	7.4
C_0	4.8	C_1	3.2	C_2	4.0	C_3	8.2	C_{4a}	8.0	C_{4b}	6.8	C_5	7.0	C_6	4.2
C_0	4.1	C_1	3.5	C_2	4.4	C_3	8.6	C_{4a}	8.0	C_{4b}	8.0	C_5	6.0	C_6	4.0
C_0	3.0	C_1	3.8	C_2	6.6	C_3	5.2	C_{4a}	9.0			C_5	9.0	C_6	4.1
C_0	3.5	C_1	2.9	C_2	8.0	C_3	4.4	C_{4a}	6.0			C_5	8.0		
C_0	3.0	C_1	3.1	C_2	5.0							C_5	8.0		
C_0	3.2	C_1	3.5												
C_0	2.4	C_1	3.4												
C_0	2.2	C_1	1.5												
C_0	5.8	C_1	2.3												
C_0	5.5	C_1	4.0												
		C_1	2.0												
		C_1	2.3												
		C_1	3.2												
		C_1	2.2												
Median	3.4	Median	3.2	Median	4.4	Median	6.2	Median	8.0	Median	5.2	Median	7.3	Median	6.0
LQ	3.0	LQ	2.8	LQ	4.0	LQ	4.6	LQ	6.3	LQ	4.8	LQ	6.0	LQ	4.1
UQ	4.1	UQ	3.6	UQ	6.0	UQ	8.1	UQ	8.8	UQ	7.1	UQ	8.1	UQ	6.3

Height of Subjects in cm (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	170.0	C_1	170.2	C_2	182.9	C_3	160.0	C_{4a}	180.3	C_{4b}	162.6	C_5	162.6	C_6	157.5
C_0	170.0	C_1	167.6	C_2	160.0	C_3	167.6	C_{4a}	175.3	C_{4b}	182.9	C_5	175.3	C_6	180.3
C_0	164.0	C_1	190.5	C_2	170.2	C_3	167.6	C_{4a}	167.6	C_{4b}	152.4	C_5	180.3	C_6	182.9
C_0	164.0	C_1	168.0	C_2	188.0	C_3	160.0	C_{4a}	180.3	C_{4b}	157.5	C_5	167.6	C_6	177.8
C_0	180.0	C_1	168.0	C_2	188.0	C_3	170.2	C_{4a}	167.6	C_{4b}	167.6	C_5	170.2	C_6	180.3
C_0	180.0	C_1	160.0	C_2	180.3	C_3	170.2	C_{4a}	180.3	C_{4b}	152.4	C_5	152.4	C_6	170.2
C_0	180.3	C_1	176.0	C_2	177.8	C_3	152.4	C_{4a}	170.2	C_{4b}	175.3	C_5	160.0	C_6	170.2
C_0	180.3	C_1	176.0	C_2	162.6	C_3	152.4	C_{4a}	165.1	C_{4b}	175.3	C_5	182.9	C_6	182.9
		C_1	167.6	C_2	152.4	C_3	167.6	C_{4a}	165.1	C_{4b}	154.9	C_5	190.5	C_6	182.9
		C_1	167.6	C_2	185.4	C_3	160.0	C_{4a}	180.3	C_{4b}	154.9	C_5	162.6	C_6	170.2
						C_3	170.2	C_{4a}	182.9						
								C_{4a}	182.9						
								C_{4a}	185.4						
Median	**175.0**	**Median**	**168.0**	**Median**	**179.1**	**Median**	**167.6**	**Median**	**180.3**	**Median**	**160.1**	**Median**	**168.9**	**Median**	**179.1**
LQ	**168.5**	**LQ**	**167.6**	**LQ**	**164.5**	**LQ**	**160.0**	**LQ**	**167.6**	**LQ**	**154.9**	**LQ**	**162.6**	**LQ**	**170.2**
UQ	**180.1**	**UQ**	**174.6**	**UQ**	**184.8**	**UQ**	**168.9**	**UQ**	**180.3**	**UQ**	**173.4**	**UQ**	**179.1**	**UQ**	**182.3**

Weight of Subjects in Kg (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	75.0	C_1	59.0	C_2	95.3	C_3	65.3	C_{4a}	96.2	C_{4b}	77.1	C_5	66.2	C_6	69.9
C_0	75.0	C_1	57.2	C_2	65.3	C_3	101.7	C_{4a}	69.9	C_{4b}	127.0	C_5	69.9	C_6	75.3
C_0	51.5	C_1	90.0	C_2	59.0	C_3	77.6	C_{4a}	57.2	C_{4b}	50.8	C_5	96.2	C_6	127.0
C_0	51.5	C_1	70.0	C_2	86.2	C_3	54.0	C_{4a}	69.9	C_{4b}	69.9	C_5	101.7	C_6	87.0
C_0	60.0	C_1	70.0	C_2	86.2	C_3	75.3	C_{4a}	62.0	C_{4b}	62.0	C_5	82.6	C_6	86.2
C_0	60.0	C_1	54.0	C_2	69.9	C_3	75.3	C_{4a}	86.2	C_{4b}	92.5	C_5	92.5	C_6	75.3
C_0	76.2	C_1	60.0	C_2	87.0	C_3	92.5	C_{4a}	75.3	C_{4b}	75.3	C_5	66.2	C_6	82.6
C_0	76.2	C_1	60.0	C_2	77.1	C_3	92.5	C_{4a}	57.2	C_{4b}	75.3	C_5	95.3	C_6	77.1
		C_1	77.6	C_2	50.8	C_3	76.2	C_{4a}	57.2	C_{4b}	95.3	C_5	90.0	C_6	77.1
		C_1	76.2	C_2	75.8	C_3	66.2	C_{4a}	75.3	C_{4b}	95.3	C_5	66.2	C_6	84.4
						C_3	84.4	C_{4a}	88.0						
								C_{4a}	88.0						
								C_{4a}	75.8						
Median	**67.5**	**Median**	**65.0**	**Median**	**76.5**	**Median**	**76.2**	**Median**	**75.3**	**Median**	**76.2**	**Median**	**86.3**	**Median**	**79.9**
LQ	**57.9**	**LQ**	**59.3**	**LQ**	**66.5**	**LQ**	**70.8**	**LQ**	**62.0**	**LQ**	**71.3**	**LQ**	**67.1**	**LQ**	**75.8**
UQ	**75.3**	**UQ**	**74.7**	**UQ**	**86.2**	**UQ**	**88.5**	**UQ**	**86.2**	**UQ**	**94.6**	**UQ**	**94.6**	**UQ**	**85.8**

Body Surface Area m^2 (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	1.9	C_1	1.7	C_2	2.2	C_3	1.7	C_{4a}	2.2	C_{4b}	1.8	C_5	1.7	C_6	1.7
C_0	1.9	C_1	1.6	C_2	1.7	C_3	2.1	C_{4a}	1.8	C_{4b}	2.5	C_5	1.8	C_6	1.9
C_0	1.5	C_1	2.2	C_2	1.7	C_3	1.9	C_{4a}	1.6	C_{4b}	1.5	C_5	2.2	C_6	2.5
C_0	1.5	C_1	1.8	C_2	2.1	C_3	1.6	C_{4a}	1.9	C_{4b}	1.7	C_5	2.1	C_6	2.1
C_0	1.8	C_1	1.8	C_2	2.1	C_3	1.9	C_{4a}	1.7	C_{4b}	1.7	C_5	1.9	C_6	2.1
C_0	1.8	C_1	1.6	C_2	1.9	C_3	1.9	C_{4a}	2.1	C_{4b}	1.9	C_5	1.9	C_6	1.9
C_0	2.0	C_1	1.7	C_2	2.1	C_3	1.9	C_{4a}	1.9	C_{4b}	1.9	C_5	1.7	C_6	1.9
C_0	2.0	C_1	1.7	C_2	1.8	C_3	1.9	C_{4a}	1.6	C_{4b}	1.9	C_5	2.2	C_6	2.0
		C_1	1.9	C_2	1.5	C_3	1.9	C_{4a}	1.6	C_{4b}	1.9	C_5	2.2	C_6	2.0
		C_1	1.9	C_2	2.0	C_3	1.7	C_{4a}	1.9	C_{4b}	1.9	C_5	1.7	C_6	2.0
						C_3	2.0	C_{4a}	2.1						
								C_{4a}	2.1						
								C_{4a}	2.0						
Median	**1.8**	**Median**	**1.8**	**Median**	**1.9**	**Median**	**1.9**	**Median**	**1.9**	**Median**	**1.9**	**Median**	**1.9**	**Median**	**2.0**
LQ	**1.7**	**LQ**	**1.7**	**LQ**	**1.7**	**LQ**	**1.8**	**LQ**	**1.7**	**LQ**	**1.7**	**LQ**	**1.7**	**LQ**	**1.9**
UQ	**1.9**	**UQ**	**1.8**	**UQ**	**2.1**	**UQ**	**1.9**	**UQ**	**2.1**	**UQ**	**1.9**	**UQ**	**2.1**	**UQ**	**2.0**

Clinical Scoring of Venous Dysfunction by the CEAP method (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)															
Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.0	C_1	0.0	C_2	2.0	C_3	3.0	C_{4a}	4.0	C_{4b}	8.0	C_5	8.0	C_6	13.0
C_0	0.0	C_1	0.0	C_2	1.0	C_3	1.0	C_{4a}	3.0	C_{4b}	8.0	C_5	7.0	C_6	7.0
C_0	0.0	C_1	0.0	C_2	0.0	C_3	3.0	C_{4a}	3.0	C_{4b}	8.0	C_5	7.0	C_6	12.0
C_0	0.0	C_1	0.0	C_2	1.0	C_3	1.0	C_{4a}	4.0	C_{4b}	7.0	C_5	9.0	C_6	4.0
C_0	0.0	C_1	0.0	C_2	1.0	C_3	1.0	C_{4a}	4.0	C_{4b}	7.0	C_5	9.0	C_6	13.0
C_0	0.0	C_1	0.0	C_2	2.0	C_3	2.0	C_{4a}	4.0	C_{4b}	8.0	C_5	10.0	C_6	12.0
C_0	0.0	C_1	0.0	C_2	2.0	C_3	2.0	C_{4a}	2.0	C_{4b}	7.0	C_5	10.0	C_6	14.0
C_0	0.0	C_1	0.0	C_2	2.0	C_3	2.0	C_{4a}	4.0	C_{4b}	7.0	C_5	10.0	C_6	12.0
		C_1	1.0	C_2	1.0	C_3	3.0	C_{4a}	4.0	C_{4b}	7.0	C_5	8.0	C_6	11.0
		C_1	0.0	C_2	1.0	C_3	2.0	C_{4a}	3.0	C_{4b}	7.0	C_5	10.0	C_6	14.0
						C_3	1.0	C_{4a}	5.0						
								C_{4a}	5.0						
								C_{4a}	5.0						
Median	**0.0**	**Median**	**0.0**	**Median**	**1.0**	**Median**	**2.0**	**Median**	**4.0**	**Median**	**7.0**	**Median**	**9.0**	**Median**	**12.0**
LQ	**0.0**	**LQ**	**0.0**	**LQ**	**1.0**	**LQ**	**1.0**	**LQ**	**3.0**	**LQ**	**7.0**	**LQ**	**8.0**	**LQ**	**11.3**
UQ	**0.0**	**UQ**	**0.0**	**UQ**	**2.0**	**UQ**	**2.5**	**UQ**	**4.0**	**UQ**	**8.0**	**UQ**	**10.0**	**UQ**	**13.0**

Disability Scoring of Venous Dysfunction by the CEAP method (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.0	C_1	0.0	C_2	1.0	C_3	1.0	C_{4a}	1.0	C_{4b}	2.0	C_5	2.0	C_6	2.0
C_0	0.0	C_1	0.0	C_2	1.0	C_3	0.0	C_{4a}	0.0	C_{4b}	1.0	C_5	1.0	C_6	2.0
C_0	0.0	C_1	0.0	C_2	0.0	C_3	1.0	C_{4a}	1.0	C_{4b}	2.0	C_5	1.0	C_6	3.0
C_0	0.0	C_1	0.0	C_2	1.0	C_3	1.0	C_{4a}	1.0	C_{4b}	1.0	C_5	2.0	C_6	2.0
C_0	0.0	C_1	0.0	C_2	1.0	C_3	0.0	C_{4a}	1.0	C_{4b}	1.0	C_5	1.0	C_6	1.0
C_0	0.0	C_1	0.0	C_2	1.0	C_3	1.0	C_{4a}	1.0	C_{4b}	2.0	C_5	2.0	C_6	2.0
C_0	0.0	C_1	0.0	C_2	1.0	C_3	1.0	C_{4a}	0.0	C_{4b}	2.0	C_5	2.0	C_6	1.0
C_0	0.0	C_1	0.0	C_2	1.0	C_3	1.0	C_{4a}	1.0	C_{4b}	2.0	C_5	2.0	C_6	2.0
		C_1	0.0	C_2	0.0	C_3	1.0	C_{4a}	1.0	C_{4b}	2.0	C_5	1.0	C_6	2.0
		C_1	0.0	C_2	1.0	C_3	1.0	C_{4a}	1.0	C_{4b}	2.0	C_5	2.0	C_6	2.0
						C_3	0.0	C_{4a}	2.0						
								C_{4a}	2.0						
								C_{4a}	1.0						
Median	**0.0**	**Median**	**0.0**	**Median**	**1.0**	**Median**	**1.0**	**Median**	**1.0**	**Median**	**2.0**	**Median**	**2.0**	**Median**	**2.0**
LQ	**0.0**	**LQ**	**0.0**	**LQ**	**1.0**	**LQ**	**0.5**	**LQ**	**1.0**	**LQ**	**1.3**	**LQ**	**1.0**	**LQ**	**2.0**
UQ	**0.0**	**UQ**	**0.0**	**UQ**	**1.0**	**UQ**	**1.0**	**UQ**	**1.0**	**UQ**	**2.0**	**UQ**	**2.0**	**UQ**	**2.0**

Anatomical Scoring of Venous Dysfunction by the CEAP method (Data with Median values and Lower-Quartile / Upper-Quartile Ranges)

Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data	Group	Data
C_0	0.0	C_1	1.0	C_2	2.0	C_3	3.0	C_{4a}	1.0	C_{4b}	2.0	C_5	2.0	C_6	4.0
C_0	0.0	C_1	1.0	C_2	3.0	C_3	3.0	C_{4a}	4.0	C_{4b}	5.0	C_5	3.0	C_6	5.0
C_0	0.0	C_1	1.0	C_2	2.0	C_3	3.0	C_{4a}	3.0	C_{4b}	6.0	C_5	3.0	C_6	5.0
C_0	0.0	C_1	1.0	C_2	3.0	C_3	4.0	C_{4a}	3.0	C_{4b}	3.0	C_5	2.0	C_6	4.0
C_0	0.0	C_1	1.0	C_2	2.0	C_3	3.0	C_{4a}	5.0	C_{4b}	4.0	C_5	6.0	C_6	3.0
C_0	0.0	C_1	1.0	C_2	2.0	C_3	4.0	C_{4a}	2.0	C_{4b}	4.0	C_5	5.0	C_6	2.0
C_0	0.0	C_1	1.0	C_2	2.0	C_3	2.0	C_{4a}	1.0	C_{4b}	2.0	C_5	3.0	C_6	6.0
C_0	0.0	C_1	1.0	C_2	2.0	C_3	2.0	C_{4a}	4.0	C_{4b}	2.0	C_5	6.0	C_6	5.0
		C_1	0.0	C_2	1.0	C_3	3.0	C_{4a}	3.0	C_{4b}	2.0	C_5	4.0	C_6	2.0
		C_1	1.0	C_2	2.0	C_3	3.0	C_{4a}	4.0	C_{4b}	2.0	C_5	2.0	C_6	4.0
						C_3	4.0	C_{4a}	7.0						
								C_{4a}	7.0						
								C_{4a}	1.0						
Median	**0.0**	**Median**	**1.0**	**Median**	**2.0**	**Median**	**3.0**	**Median**	**3.0**	**Median**	**2.5**	**Median**	**3.0**	**Median**	**4.0**
LQ	**0.0**	**LQ**	**1.0**	**LQ**	**2.0**	**LQ**	**3.0**	**LQ**	**2.0**	**LQ**	**2.0**	**LQ**	**2.3**	**LQ**	**3.3**
UQ	**0.0**	**UQ**	**1.0**	**UQ**	**2.0**	**UQ**	**3.5**	**UQ**	**4.0**	**UQ**	**4.0**	**UQ**	**4.8**	**UQ**	**5.0**

SECTION VI

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